Copy for the designated Office (DO/ US)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

To:

FLYNN, Kerry, A.
Biogen, Inc.
14 Cambridge Center
Cambridge, MA 02142
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 09 October 1996 (09.10.96)	
Applicant's or agent's file reference B189PCT	REPLY DUE see paragraph 1 below
International application No. PCT/US96/10664	International filing date *(day/month/year)* 21 June 1996 (21.06.96)
Applicant BIOGEN, INC. KARPUSAS, Mihail, N. et al	

1. [ ] REPLY DUE within ____ months/days from the above date of mailing

[ ] NO REPLY DUE, however, see below _____

[X] IMPORTANT COMMUNICATION

[ ] INFORMATION ONLY

2. COMMUNICATION:

Please be informed, in respect of the above-international application, that, consequent to the applicant's timely filed request for rectification of an obvious error according to PCT Rule 91, the receiving Office has informed the International Bureau that the rectification is to be autorized as requested by the applicant.

The priority claim (s) in Box VI of the request form (form PCT RO/101) should read :

Country	Filing Date	Application No.
US	22 June 1995 (22.06.95)	60/000,448

instead of :

US	21 June 1996 (21.06.96)	60/000,448

Copies : The Designated Offices concerned
The Receiving Office (RO/US)
The International Searching Authority (ISA/EP)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No. (41-22) 740.14.35

Authorized officer
J. Rey-Millet
Telephone No. (41-22) 730.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 February 1997 (14.02.97)	
International application No. PCT/US96/10664	**Applicant's or agent's file reference** B189PCT
International filing date (day/month/year) 21 June 1996 (21.06.96)	**Priority date** (day/month/year) 22 June 1995 (22.06.95)
Applicant KARPUSAS, Mihail, N. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 January 1997 (21.01.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Ann Bardini
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 730.91.11

TENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
B189PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 96/ 10664	21/06/1996	22/06/1995
Applicant		
BIOGEN, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. 1
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PC., US 96/10664

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/725 C07K14/525 C07K1/00 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 585 943 (BRISTOL-MYERS SQUIBB COMPANY) 9 March 1994 see the whole document ---	21-26,36
X	EP,A,0 555 880 (BRISTOL-MYERS SQUIBB COMAPNY) 18 August 1993 see the whole document ---	21-26,36
X	WO,A,94 17196 (IMMUNEX COPRPORATION) 4 August 1994 see the whole document ---	21-26,36
X	WO,A,93 08207 (IMMUNEX CORPORATION) 29 April 1993 see the whole document --- -/--	21-26,36

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 1996	15. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGICAL METHODS, vol. 149, no. 2, 15 June 1992, NEW YORK US, pages 655-660, XP002017167 W C FANSLOW ET AL.: "Soluble forms of CD40 inhibit biologic responses of human B cells" see the whole document ---	21-26,36
X	CHEMICAL ABSTRACTS, vol. 120, no. 3, 17 January 1994 Columbus, Ohio, US; abstract no. 28892n, M C PEITSCH & V C JONGENEEL: "A 3-D model for the CD40 ligand predicts that it is a compact trimer similar to the tumor necrosis factor" page 659; XP002017169 see abstract & INT. IMMUNOL. , vol. 5, no. 3, 1993, pages 233-238, ---	13, 21-26, 28-38
P,X	BIOCHEMISTRY, vol. 34, no. 31, 8 August 1995, EASTON, PA US, pages 9884-9892, XP002017168 J BAJORATH ET AL.: "Analysis of gp39/CD40 interactions using molecular models and site-directed mutagenesis" see the whole document ---	13, 21-26, 28-38
P,X	CHEMICAL ABSTRACTS, vol. 123, no. 23, 4 December 1995 Columbus, Ohio, US; abstract no. 311922s, M KARPUSAS ET AL.: "2A crystal structure of an extracellular fragment of human CD40 ligand" page 700; XP002017170 see abstract & STRUCTURE, vol. 3, no. 10, 1995, LONDON, pages 1031-1039, -----	1-38

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PC JS 96/10664

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-585943	09-03-94	US-A-	5540926	30-07-96
		AU-A-	4612093	10-03-94
		CA-A-	2105552	05-03-94
		FI-A-	933862	05-03-94
		HU-A-	69977	28-09-95
		JP-A-	6315383	15-11-94
		NO-A-	933126	07-03-94
		NZ-A-	248569	26-10-95
		ZA-A-	9306491	25-03-94
EP-A-555880	18-08-93	AU-A-	3298893	19-08-93
		CA-A-	2089229	15-08-93
		JP-A-	6220096	09-08-94
		NZ-A-	245898	27-04-95
		ZA-A-	9301013	20-09-93
WO-A-9417196	04-08-94	US-A-	5565321	15-10-96
		AU-A-	6231394	15-08-94
		CA-A-	2153806	04-08-94
		EP-A-	0679191	02-11-95
WO-A-9308207	29-04-93	AU-B-	661360	20-07-95
		AU-A-	3122693	21-05-93
		CA-A-	2121798	29-04-93
		EP-A-	0667901	23-08-95
		FI-A-	941837	30-05-94
		JP-T-	7504083	11-05-95
		NO-A-	941422	27-06-94

PATENT COOPERATION TREATY

REC'D 30 SEP 1997
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
B189PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US96/10664	21/06/1996	22/06/1995
International Patent Classification (IPC) or national classification and IPC		
C07K14/725		
Applicant		
BIOGEN, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 24 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/01/1997	26.09.97
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Döpfer, K-P Telephone No. (+49-89) 2399-8547

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US96/10664

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-4,6-12,14-16,18-28, 30-32,34	as originally filed			
5,5a,13,13a,17,17a, 29,29a,33,33a,35, 35a,36,36a,37,37a, 38,38a	as received on	22/08/1997	with letter of	22/08/1997

Claims, No.:

1-7,8 (part),15-17, 23-27	as originally filed			
8 (part),9-14,18-22, 28-36	as received on	22/08/1997	with letter of	22/08/1997

Drawings, sheets:

1/8-8/8	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 12 (subsequent claims should therefore be re-numbered)
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 12, 17-24, 27-34.

because:

☒ the said international application, or the said claims Nos. 12, 17-24, 27-34 relate to the following subject matter which does not require an international preliminary examination (*specify*):

cf Separate Sheet, point 3

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims..

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11, 13-16, 25, 26, 36, 37
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11, 13-16, 25, 26, 36, 37
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11, 13-16, 25, 26, 36, 37
	No:	Claims	

2. Citations and explanations

cf Separate Sheet, point 2

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

2. Non-written disclosures (Rule 70.9)

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

cf. Separate Sheet, point 4

1. Reference is made to the following documents:

   D1: EP-A-585 943
   D2: EP-A-555 880
   D3: WO-A-94/17196
   D4: WO-A-93/08207
   D5: J. immunol. Meth. **149**(2):655-660(1992)
   D6: CA **120**(3):28 892n(1994) & Int. Immunol. **5**(3):233-238(1993)

2. The prior art as cited in D1 to D6 discloses CD40 and CD40 ligands and their effects on B-cell responses. D6 itself is directed to the modeling of the three-dimensional structure of CD40L on the basis of TNF_{α} as a template in order to determine which amino acids are essential for the maintenance of its trimeric structure, and, which region(s) of CD40L could interact with its receptors.
   D1 which is considered as closest prior art discloses soluble ligands for the B-cell antigen CD40.

   The problem underlying the present application can be regarded as to provide a method to design ligands capable to interfere the CD40/CD40L interaction in order to modulate B-cell responses.

   The solution is a method to obtain CD40L in crystalline form and the use of this crystals in designing ligands which are able to affect the interaction of the B-cell antigen CD40 with its ligand CD40L.

   The method to obtain crystals of Cd40L, and in particular of the part of the outer domaine with the sequence 116-261 has not been described in the prior art. Furthermore, no hint can found for the special embodiments of the methods or the crystallographic data characterising the crystals obtained by the claimed method. Accordingly, the subject-matter of present claims 1-11, 13-16, 25, 26, 36 and 37 fulfills the requirements of novelty and inventive step (Article 33(2)(3) PCT). Industrial applicability is acknowledged (Article 33(4) PCT.

3.1 Present claims 12, 17-19, 27-34 are directed to subject-matter which are schemes

or rules to perform purely mental acts, mere presentation of information, and programs for computers. According to Rule 67(1)(i)(iii)(v)(vi) PCT, no international preliminary examination is required for these subject-matter.

3.2 The amendments filed with the letter dated 22.08.97 relating to claims 20-24 and 35 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: "*chemical entities having a molecular weight of less than 2000 daltons*".

4.1 The relative term "*a crystal ..., or a homolog thereof...*" used in claims 13-16 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

4.2 Claims 23 and 24 are dependent on claims 21 or 22 and refer to a method of these claims. But these claims are directed to chemical entities. Accordingly, these claims are not clear in the sense of Article 6 PCT (cf also point 3.2 of this report).

PATENT COOPERATION TREA

COPY

Entered Computer

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

FLYNN, Kerry, A.
BIOGEN, Inc.
14 Cambridge Center
Cambridge, Ma 02142
ETATS-UNIS D'AMERIQUE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	26.09.97
Applicant's or agent's file reference: B189PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US96/10664	21/06/1996	22/06/1995

Applicant
BIOGEN, INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

BIOGEN, INC IP DEPT.
OCT 17 1997
REFERRED TO KAF
NOTED BY

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	DA ROCHA, O. Tel. (+49-89) 2399-8101

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference B189PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
International application No. PCT/US96/10664	International filing date *(day/month/year)* 21/06/1996	Priority date *(day/month/year)* 22/06/1995
International Patent Classification (IPC) or national classification and IPC C07K14/725		
Applicant BIOGEN, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 24 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/01/1997	26. 09. 97
Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Döpfer, K-P Telephone No. (+49-89) 2399-8547

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US96/10664

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-4,6-12,14-16,18-28, 30-32,34	as originally filed			
5,5a,13,13a,17,17a, 29,29a,33,33a,35, 35a,36,36a,37,37a, 38,38a	as received on	22/08/1997	with letter of	22/08/1997

Claims, No.:

1-7,8 (part),15-17, 23-27	as originally filed			
8 (part),9-14,18-22, 28-36	as received on	22/08/1997	with letter of	22/08/1997

Drawings, sheets:

1/8-8/8	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 12, 17-24, 27-34.

because:

☒ the said international application, or the said claims Nos. 12, 17-24, 27-34 relate to the following subject matter which does not require an international preliminary examination (*specify*):

cf Separate Sheet, point 3

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims..

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11, 13-16, 25, 26, 36, 37
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11, 13-16, 25, 26, 36, 37
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11, 13-16, 25, 26, 36, 37
	No:	Claims	

2. Citations and explanations

cf Separate Sheet, point 2

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

2. Non-written disclosures (Rule 70.9)

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

cf. Separate Sheet, point 4

1. Reference is made to the following documents:

   D1: EP-A-585 943
   D2: EP-A-555 880
   D3: WO-A-94/17196
   D4: WO-A-93/08207
   D5: J. immunol. Meth. **149**(2):655-660(1992)
   D6: CA **120**(3):28 892n(1994) & Int. Immunol. **5**(3):233-238(1993)

2. The prior art as cited in D1 to D6 discloses CD40 and CD40 ligands and their effects on B-cell responses. D6 itself is directed to the modeling of the three-dimensional structure of CD40L on the basis of TNF_{α} as a template in order to determine which amino acids are essential for the maintenance of its trimeric structure, and, which region(s) of CD40L could interact with its receptors.
   D1 which is considered as closest prior art discloses soluble ligands for the B-cell antigen CD40.

   The problem underlying the present application can be regarded as to provide a method to design ligands capable to interfere the CD40/CD40L interaction in order to modulate B-cell responses.

   The solution is a method to obtain CD40L in crystalline form and the use of this crystals in designing ligands which are able to affect the interaction of the B-cell antigen CD40 with its ligand CD40L.

   The method to obtain crystals of Cd40L, and in particular of the part of the outer domaine with the sequence 116-261 has not been described in the prior art. Furthermore, no hint can found for the special embodiments of the methods or the crystallographic data characterising the crystals obtained by the claimed method. Accordingly, the subject-matter of present claims 1-11, 13-16, 25, 26, 36 and 37 fulfills the requirements of novelty and inventive step (Article 33(2)(3) PCT). Industrial applicability is acknowledged (Article 33(4) PCT.

3.1 Present claims 12, 17-19, 27-34 are directed to subject-matter which are schemes

or rules to perform purely mental acts, mere presentation of information, and programs for computers. According to Rule 67(1)(i)(iii)(v)(vi) PCT, no international preliminary examination is required for these subject-matter.

3.2 The amendments filed with the letter dated 22.08.97 relating to claims 20-24 and 35 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: "*chemical entities having a molecular weight of less than 2000 daltons*".

4.1 The relative term "*a crystal ..., or a homolog thereof...*" used in claims 13-16 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

4.2 Claims 23 and 24 are dependent on claims 21 or 22 and refer to a method of these claims. But these claims are directed to chemical entities. Accordingly, these claims are not clear in the sense of Article 6 PCT (cf also point 3.2 of this report).

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to crystals of CD40L or crystals of fragments of CD40L, of sufficient size and quality to obtain useful information about the properties of CD40L and molecules or complexes which may associate with CD40L or CD40. The claimed invention provides the three-dimensional crystal structure of the Gly116 to Leu261 fragment of CD40L, which can be used to identify binding sites to solve the structure of unknown crystals, to provide mutants having desirable binding properties, and ultimately, to design, characterize, or identify molecules or chemical entities capable of interfering with the interaction between CD40 and CD40L.

Additional features and advantages of the invention will be set forth in the description which follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and other advantages of the invention will be realized and attained by the compositions and methods particularly pointed out in the written description and claims hereof, as well as in the appended drawings.

To achieve these and other advantages, and in accordance with the purpose of the invention, as embodied and broadly described herein, the invention relates to a crystal of CD40L. More particularly, the invention relates to a crystal formed by a functional fragment of the extracellular domain of sCD40L(116-261), wherein the crystal has cell constants a=b=77.17 x 10^{-10} M (Å), c= 90.46 x 10^{-10} M

(Å), $\alpha = \beta = 90°$, $\gamma = 120°$, and a space group of R3, and equivalents of that crystal. The claimed crystals of CD40L are substantially described by the structural coordinates identified in Table 1. The claimed crystals in certain embodiments are characterized by a binding site moiety comprising Ile127,

coordinates derived from mathematical equations related to the patterns obtained on diffraction of a monochromatic beam of X-rays by the atoms (scattering centers) of molecule in crystal form. The diffraction data are used to calculate an electron density map of the repeating units of the crystal. Those skilled in the art will understand that the data obtained are dependent upon the particular system used, and hence, different coordinates may in fact describe the same crystal if such coordinates define substantially the same relationship as those described herein. The electron density maps are used to establish the positions of the individual atoms within the unit cell of the crystal.

Those of skill in the art understand that a set of structural coordinates determined by X-ray crystallography is not without standard error. Table 1 is the atomic coordinates of sCD40L(116-261). For the purpose of this invention, any set of structural coordinates of CD40L(116-261) that have a root mean square deviation of equivalent protein backbone atoms of less than about 2 x 10^{-10} M (Å) when superimposed--- using backbone atoms-- on the structural coordinates in Table 1 shall be considered identical. Preferably the deviation is less than about 1 x 10^{-10} M (Å) and more preferably less than about 0.5 x 10^{-10} M (Å).

The term "heavy atom derivatization" refers to a method of producing a chemically modified form of a crystallized CD40 ligand. In practice, a crystal is soaked in a solution containing heavy metal atom salts, or organometallic compounds, e.g., lead chloride, gold thiomalate, thimerosal or uranyl acetate, which can diffuse through the crystal and

bind to the surface of the protein. The location of the bound heavy metal atom(s) can be determined by X-ray diffraction analysis of the soaked crystal. This information can be used to generate the phase information used to construct the three-dimensional structure of the molecule.

crystals of a fragment of CD40 ligand(116-261) having unit cells which are rhombohedral, and having the following dimensions a=b=77.17 x 10^{-10} M (Å) and c=90. 46 x 10^{-10} M (Å), $\alpha=\beta=$ 90 x 10^{-10} M (Å) and γ = 120 x 10^{-10} M (Å). Almost all of the residues of CD40 ligand fragment, except for residues 116-119 of the N terminus, are well defined in the final electron density map shown in Figure 1. There are areas of weak density for residues 182-186 and 210-220. The current model consists of 142 amino acid residues and 95 water molecules with a crystallographic R factor of 21.8 % and an R_{free} of 29.1% for data between 7.5 x 10^{-10} M (Å) and 2 x 10^{-10} M (Å). The Ramachandran diagram shows that 140 out of the 142 amino acid residues have (ϕ,ψ) angles within the allowed regions. The exceptions are residue Cys218, which is involved in the formation of a disulfide bridge, and Lys143.

CD40 ligand folds as a sandwich of two β sheets with jellyroll or Greek key topology (Figure 3). The dimensions of the molecule are 25 x 10^{-10} M (Å) x 30 x 10^{-10} M (Å) x 50 x 10^{-10} M (Å). The overall fold is similar to that of TNF-α and LT-α. The notation used herein is that described in Eck et.al., "The Structure of Human Lymphotoxin", J. Biol. Chem., 267, 2119-2112, for the β strands and other structural features. One β sheet consists of strands A"AHCF, and the other of strands B'BGDE. To assess the degree of structural similarity between TNF-α, LT-α and CD40 ligand, the sequences were aligned to maximize the overlap of equivalent β strand residues. The equivalent Cα atoms from the β strands were then used to superimpose the

molecular structures. The root mean square (rms) positional deviation of 86 equivalent Cα atoms of superimposed TNF-α and CD40L molecules is 1.10×10^{-10} M (Å). In the case of the LT/CD40L pair, the rms deviation for the 100 equivalent Cα atoms is 1.03×10^{-10} M (Å). The positions of the residues are highly conserved in the core and β strand regions, but differ significantly in certain loops, such as the AA", CD and EF loops. An alignment of the TNF-α, LT-α

and the mutation will be clinically undetectable. This behavior is consistent with the fact that, in the LTα-receptor complex structure, as many as 38 LTα residues are involved in the binding interface. The interface is quite extensive (520 10^{-10} M^2 ($Å^2$)) and is presumably of similar dimensions in the CD40L-CD40 complex. It is expected, therefore, that each residue only contributes a small fraction of the binding energy. However, the interaction of the human growth hormone with its receptor has also been shown to involve a large surface area, yet relatively few key residues contribute most of the binding energy.

Additionally, the claimed invention is useful for the optimization of potential small molecule drug candidates. Thus, the claimed crystal structures can be also be used to obtain information about the crystal structures of complexes of the CD40 ligand and small molecule inhibitors. For example, if the small molecule inhibitor is co-crystallized with CD40 ligand, then the crystal structure of the complex can be solved by molecular replacement using the known coordinates of CD40 ligand for the calculation of phases. Such information is useful, for example, for determining the nature of the interaction between the CD40 ligand and the small molecule inhibitor, and thus, may suggest modifications which would improve binding characteristics such as affinity, specificity and kinetics.

The detrimental effects of several HIGMS mutations on CD40L appear to be caused by destabilizing its structure. For example, Trp140 is a large hydrophobic residue buried inside the protein and removal of its side chain in the HIGMS mutations Trp140→Gly and Trp140→Arg will obviously

lead to destabilization of the structure. The valine affected by the Val126→Ala mutation also participates in forming the hydrophobic core. Leu155 is not completely buried but lies in the middle of β strand B. Introduction of proline at this position, as in the Leu155→Pro HIGMS

C. EXAMPLES

1. Determination of Crystal Structure of sCD40L(116-261)

A. Crystallization

Buffer chemicals were purchased from Fisher (Boston, MA). Crystallization condition screenings were done with the Crystal Screen™ kit from Hampton Research (Riverside, CA). A soluble fragment of the extracellular domain of human CD40 ligand containing amino acid residues Gly116 to the C-terminal residue Leu261 was produced in soluble form and purified as follows:

The gene encoding the sCD40L(116-261) sequence of amino acids G116-L261 was cloned into the *Pichia pastoris* expression vector pWS106 and the sCD40L(116-261) protein was expressed by using standard protocols. Peitsch et al., "A B-D Model for the CD40 Ligand Predicts That it is a Compact Trimer Similar to the Tumor Necrosis Factors.", Int. Immunol. 5, 233-238, (1993). pWS106 is a variant of vector pPIC9 (Invitrogen) with the NcoI site at 3634 nt in the 3'-AOX1 flanking region deleted by site directed mutagenesis. The cells were lysed and the medium was dialyzed overnight with 20mM Tris-HCl, pH 6.8 and loaded onto an SP (Pharmacia) column. The bound sCD40L was eluted by 2xPBS, pH 7.2 and buffer-exchanged to 1xPBS.

The protein stock was made available as a solution of 8 mg/ml sCD40L in PBS (20.44 g/ℓ Na_2HPO_4, 7.73 g/ℓ Na_2HPO_4-H_2O, 87.66 g/lt NaCl) buffer. Crystals were grown by the vapor diffusion method. (see Jancarik et.al.). In order to find conditions of crystallization, an incomplete factorial screen was set up. In a typical experiment, protein

solution was mixed with an equal volume of reservoir solution and a drop of the mixture was suspended under a glass cover slip over the reservoir solution. Crystals were grown out of 1.4 M Na Citrate, 50 mM Na, Hepes pH 7.5 reservoir solution. The crystals are shaped as cubes, are

gradually in a cryoprotectant solution of 20% glycerol, 1.2 M Na Citrate , 50 mM Na Hepes pH 7.5, mounted on a loop and immediately frozen in a -150 °C liquid nitrogen gas stream. The technique of freezing the crystals essentially immortalizes them and produced a much higher quality data set. A native X-ray data set up to 1.75 x 10^{-10} M (Å) resolution was collected by using a Nicolet/Siemens multiwire area detector (Siemens, Inc.). The data were integrated and reduced using BUDDHA (25) and the CCP4 program package (The SERC (UK) Collaborative Computing Project No 4, Daresbury Laboratory, UK 1979). The data collection required about 5 days.

Data processing suggested a rhombohedral unit cell with approximate cell dimensions a=b=c=55 x 10^{-10} M (Å) and $\alpha=\beta=\gamma=91$. To assist calculations, a hexagonal unit cell is defined instead with dimensions a=b=77.17 x 10^{-10} M (Å), c=90.46 x 10^{-10} M (Å), $\gamma=120$ degrees. Merging of data suggested that the space group is R3. If space group R3 is assumed, then R_{merge} is 6.7%. Assuming space group R32, R_{merge} is 16.6%. Table 1 contains information for the data set obtained.

Calculation of the Matthews volume gives VM= 533,610 A^3/Z*MW=2.97 assuming Z=9 (#of asymmetric units in unit cell) and MW=20,000 daltons. Eck et.al., J. Biol.Chem. 267, 2119-2112 (1992), specifically incorporated herein by reference. Thus, it was not clear initially whether there were one or two monomers in the asymmetric unit.

C. Molecular replacement

All subsequent molecular replacement and refinement computing was done with the XPLOR program package. Molecular graphics manipulations were done with QUANTA (Molecular Simulations, Inc.) software. Both XPLOR and QUANTA were run on a Silicon Graphics Indigo2 computer workstation.

To investigate whether non-crystallographic symmetry was present, the self rotation function was calculated with 8-4 x 10^{-10} M (Å) data. A very strong peak was found for ϕ=90°, ψ=90°,

κ=180°, which corresponds to the crystallographic 3-fold axis. This coincides with the 3-fold axis of the trimer of SCD40L. Also, less strong peaks appeared for ϕ=0°, ψ=30°, κ=180° and ϕ=0°, ψ=90°, κ=180° and ϕ=0°, ψ=150°, κ=180° corresponding to 2-fold axes perpendicular to the 3-fold. None of these axes corresponded to non-crystallographic symmetry.

A 3-dimensional model of the human sCD40L was constructed by using the murine CD40L model as a framework, using QUANTA protein homology modeling software. The model was used as a probe for molecular replacement calculations.

Calculation of the cross rotation function by using a 2.5° angle grid and 8-4 x 10^{-10} M (Å) data produced a strong peak 4.5σ above the mean, at Euler angles θ_1=234.5°, θ_2=5.0°, θ_3=234.5°. Rotation of the model according to that solution and subsequent generation of symmetry-related molecules corresponding to the 3-fold crystallographic symmetry produces a trimer with the 3-fold axis parallel to the z axis, as expected. The exact rotation solution was found with Patterson correlation refinement. No significant second peak was observed in the rotation function.

In order to conduct the translation search, the model probe was rotated according to the first peak of the rotation search and translated so that the center of mass of the trimer would lie on the z axis. This was assumed to be close to the expected position since it appeared that the 3-fold axis of the trimer lay on the z axis of the unit cell. Initial attempts to find a clear peak in the translation function on the xy plane failed. Trimmed model

probes, which contained mostly core residues whose structure was more likely to be conserved among different members of the TNF family, produced translation functions with a clustering of peaks at locations close to the expected ones. One of those trimmed models containing all backbone atoms as well as side chain atoms for residues 8-12, 53-62, 90-93,

110-114 and 141-146 produced a peak with a correlation coefficient of 3.3σ above the mean at x=0.053, y=0.053. This peak was the highest on the list of peaks and was close to the expected position.

By generating symmetry related molecules and displaying them with computer graphics it was found that they packed satisfactorily in the unit cell and that there was not enough space for a second molecule. Thus, we concluded that there was only one molecule in the asymmetric unit. The existence of 2-fold symmetry in the self rotation function apparently is a consequence of internal symmetry within the molecule, possibly related to the high content of parallel strands. In fact, the self rotation function calculated from structure factors derived from the model shows peaks corresponding to 2-fold symmetry.

D. Model building and crystallographic refinement

The param19.pro stereochemical parameter set of XPLOR package was used for all refinement calculations. The partial model that was used to find the solution of the translation function was subjected to rigid body refinement by using 7.5-2.5 x 10^{-10} M (Å) data. The R and R_{free} (29) factors after the initial rigid body refinement were 49.9% and 51.4% respectively. The test data set used for the calculation of R_{free} contained 10% of the data. The partial model was subjected to 40 steps of conventional positional refinement and a cycle of simulated annealing with an initial temperature of T=2500 K. The R and R_{free} factors dropped to 31.2% and 43.1% respectively. To reduce model bias, a partial model was used for map calculation and refinement. The resolution range used was 7.5-2.5 x 10^{-10} M

(Å). Simulated annealing omit maps (30) calculated by consequently omitting 10% of the model each time showed which parts of the model could be used for phasing. Thus, the model was modified to include only residues sufficiently well defined in annealed omit maps. The initial model included 815 atoms out of a

total of 1374 atoms of the complete model. $3F_o - 2F_c$ maps were used for cycles of model building and refinement. Typically, cycles consisted of model building, positional refinement and B-factor refinement. Occasionally simulated annealing was performed. As the phases improved, more atoms were added into the model. Initially, grouped B-factors were assigned for each strand (one for main chain and the one for side chain atoms). Later, grouped B-factors and finally individual atomic B-factors where refined for each residue. Only manual structure modifications that resulted in lower R_{free} after refinement were accepted. When R and R_{free} reached 31.1% and 36.4% respectively, the resolution was extended to 2.25 x 10^{-10} M (Å) and finally to 2 x 10^{-10} M (Å). Water molecules were added by using the X-solvate utility of QUANTA 4.1. Both occupancies and temperature factors were refined for the water molecules. Figure 8 summarizes information regarding crystallographic data and refinement. Table 1 lists the atomic coordinates of sCD40L(116-261).

The coordinates of the crystal structure of a sCD40L may be used in the structure-based design of small molecule inhibitors of CD40L, computational drug design and iterative structure optimization.

a. Computational drug design

Small molecule inhibitors can be designed using computational approaches. These approaches are also known as de novo drug design. In brief, the crystal structure coordinates of the sCD40L are the input for a computer program, such as DOCK. Programs such as DOCK output a list of small molecule structures that are expected to bind to

CD40L. These molecules can then be screened by biochemical assays for CD40L binding.

Typically, biochemical assays that screen molecules for their ability to bind to CD40L are competition-type assays. In such assays, the molecule is added to the assay solution and the degree of inhibition is

solution is about 4 to about 10.

9. The method of claim 8 wherein the pH is about 7.5.

10. The method of claim 1 wherein step d) is by vapor diffusion crystallization, batch crystallization, liquid bridge crystallization or dialysis crystallization.

11. A crystal formed by a functional fragment of the extracellular domain of CD40 ligand having approximately the following cell constants: a+b=77.17 x 10^{-10} M (Å), c= 90.46 x 10^{-10} M (Å), $\alpha = \beta = 90°$, $\gamma = 120°$, and a space group of R3.

12. Deleted.

13. A machine readable data storage medium comprising a data storage material encoded with machine readable data which, when read by an appropriate machine, is capable of displaying a three dimensional representation of a crystal of a molecule or molecular complex comprising a fragment of CD40L having a binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

14. A crystal of CD40 ligand (116-261) according to claim 11, or a homolog thereof, wherein said crystal comprises a binding site, said binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

15. A crystal according to claim 11 or a homolog thereof wherein said the crystal comprises Arg207 in close proximity to at least two hydrophobic residues.

19. The method of claim 18 wherein the structural coordinates used in step a) are (1) substantially the same as those described in Table 1 or (2) describe substantially the same crystal as the coordinates in Table 1.

20. A method for evaluating the ability of a chemical entity to associate with CD40 ligand or CD40, a fragment of CD40 or CD40 ligand, or a complex comprising CD40 ligand, CD40, or homologs thereof, said method comprising the steps of:

a) employing computational or experimental means to perform a fitting operation between the chemical entity and said CD40 ligand or CD40, fragment or complex thereof, thereby obtaining data related to said association; and

b) analyzing the data obtained in step a) to determine the characteristics of the association between the chemical entity and said CD40 ligand or CD40, fragment or complex.

21. A chemical entity having a molecular weight of less than 2000 daltons identified by the method of claim 20, wherein said chemical entity is capable of interfering with the in vivo or in vitro association between CD40 and CD40L.

22. A chemical entity having a molecular weight of less than 2000 daltons identified by the method of claim 20, wherein said chemical entity is capable of associating

comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

23. A chemical entity having a molecular weight of less than 2000 daltons identified by the method of claim 20 wherein said chemical entity is capable of associating with CD40, and comprises a binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

29. A method of computationally or experimentally evaluating a chemical entity to obtain information about it's association with the binding site of CD40 ligand using a crystal of CD40 ligand or the structural coordinates thereof.

30. The method of claim 29 wherein the crystal has the structural coordinates described in Table 1.

31. The use of the structural coordinates of a crystal, wherein said crystal is substantially the same as the crystal of CD40 ligand described by the coordinates in Table 1.

32. The method of claim 29 wherein said crystal is a crystal according to claim 11.

33. The use of the structural coordinates of a crystal according to claim 31, to identify, characterize or design chemical entities having a desired association with a CD40 ligand, or fragment thereof.

34. The method of claim 33 further comprising the step of optimizing the binding characteristics of the chemical entity identified, characterized, or designed.

35. The method of claim 34 further comprising the step of determining the orientation of ligands in a binding site of CD40 ligand.

36. A chemical entity having a molecular weight of less than 2000 daltons identified or designed according to claim 33.

37. The use of a CD40 ligand crystal to determine binding interactions between a chemical entity and CD40 ligand.

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CRYSTALS OF FRAGMENTS OF CD40 LIGAND AND THEIR USE

The present invention relates to a crystalline form of a soluble fragment of the extracellular domain of human CD40 ligand, and more particularly, to a crystal of a CD40L fragment containing residues Gly116 to Leu261 ("sCD40L(116-261)") and its structure, obtained by x-ray diffraction. In addition, this invention relates to methods of using a crystal structure of CD40L, or a fragment thereof, and, specifically sCD40L(116-261), to design, screen, and optimize compounds that affect CD40L activity.

BACKGROUND OF THE INVENTION

The immune system consists of many complex processes, which, together, protect the body against toxic and/or pathogenic agents. Generally, an immune response is initiated when certain cells within the body recognize the toxic or pathogenic agents. The response is then communicated through any of a series of means, such as cell-cell contact or soluble mediators, to other cells involved in the immune response.

CD40 ligand ("CD40L") has been shown to mediate functional T and B cell interactions involved in the immune response. The term CD40L refers to a genus of polypeptides which are capable of binding CD40, or homologs thereof. In fact, the interaction of CD40L with CD40 expressed by B-cells is the principal molecular interaction responsible for T cell contact dependent induction of B cell growth and differentiation, to antigen specific antibody production. Binding of T-cell CD40L to its counter-receptor CD40 expressed on the surface of B lymphocytes results in pleiotropic activities, including activation of antibody isotype switching, prevention of B cell apoptosis, establishment of immunological memory, germinal center

formation in lymphoid tissues, modulation of cytokine production in activated B cells and B cell proliferation and differentiation

A soluble version of CD40L can be made from the extracellular region or fragments thereof. As used herein, the term CD40L includes soluble CD40L polypeptides lacking transmembrane and intracellular regions, homologs and analogs of CD40L or derivatives thereof. Early studies have shown that CD40L is a member of the tumor necrosis factor (TNF) family of cytokines. Other members of this family include TNF-α, LT-α (lymphotoxin-α, known also as TNF-β), LT-β, Fas ligand, CD30L and CD27L. CD40L is a membrane bound polypeptide with an extracellular region at its C terminus, a transmembrane region, and an intracellular region at the N terminus.

Several mutations of CD40L are known to cause an X-chromosome-linked severe immunodeficiency, known as Hyper-IgM syndrome ("HIGMS"). HIGMS is characterized by normal or elevated levels of IgM, but absent or low levels of IgG, IgA and IgE in serum. A murine CD40L gene "knockout" also lacks expression of IgG, IgA and IgE, similar to Hyper-IgM syndrome in man. These observations suggest that CD40 signaling is required for IgG, IgA, and IgE production, and that this function is non-redundant with other signaling/adhesion pathways. Studies show that CD40L may also play roles in certain diseases such as arthritis, lupus, multiple sclerosis, graft versus host disease, ulcerative colitis, allergies, tissue transplantation and generation of antibodies against antigenic drugs. Thus, it is apparent that interfering with the CD40-CD40L interaction has wide reaching therapeutic and diagnostic applications.

There is presently an interest in designing, identifying or obtaining potential drug candidates which would interfere with the CD40-CD40L interaction. The recent emergence of drug design to identify candidates that play a

role in a physiologically relevant biological pathway has provided a useful approach for obtaining, or designing, lead compounds for drugs.

Generally, this approach requires selecting a protein target molecule which plays a role in a physiologically relevant biological pathway. Typically, once a ligand, natural or synthesized, is found for the target molecule, it is modified or optimized to produce a candidate with the desired properties.

In order to more efficiently design or modify a ligand, it is useful to have a three-dimensional structure for the bioactive conformation of a known ligand as it binds to the target protein molecule. Furthermore, it is valuable to understand the detailed interactions of the ligand with its target protein by examining the three-dimensional structure of the protein target in complex with its known ligand. This allows the artisan to preserve the critical interactions with the protein, while modifying candidate ligands to interact more precisely with the protein, resulting in better potency and specificity.

However, the three dimensional crystal structure of the protein target is frequently unavailable due to the significant effort required to obtain crystals of sufficient size and resolution to provide accurate information regarding the structure. For example, it is time consuming and often difficult to express, purify and characterize a protein. Additionally, once the protein of sufficient purity is obtained, it must be crystallized to a size and clarity which is useful for x-ray diffraction and subsequent structure solution. Thus, although crystal structures can provide a wealth of valuable information in the field of drug design and discovery, crystals of certain biologically relevant compounds such as CD40L, are not readily available to those skilled in the art.

Furthermore, although the amino acid sequence of a

target protein or its ligand, such as CD40L, is known, this sequence information does not allow an accurate prediction of the crystal structure of the protein/ligand. Nor does the sequence information afford an understanding of the structural, conformational and chemical interactions between a ligand such as CD40L and its protein target.

Thus, there is a need for a detailed knowledge of the crystalline three-dimensional structure of the extracellular domain of CD40L, to effectively design, screen or optimize compounds capable of interfering with the CD40L -CD40 interactions. Although the binding of CD40 and its ligands has been studied, and similarities found between this system and the TNF system, the differences that exist suggest that these two ligand-receptor systems utilize spatially overlapping, but nonidentical and nonconserved sites of contact residues with different molecular determinants of binding.

Crystals of CD40L or fragments of CD40L of a size and quality such as to allow performance of x-ray diffraction studies would enable those skilled in the art to conduct studies relating to the binding properties of CD40L, as well as the binding properties of molecules or molecular complexes which may associate with CD40L or a fragment thereof.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to crystals of CD40L or crystals of fragments of CD40L, of sufficient size and quality to obtain useful information about the properties of CD40L and molecules or complexes which may associate with CD40L or CD40. The claimed invention provides the three-dimensional crystal structure of the Gly116 to Leu261 fragment of CD40L, which can be used to identify binding sites to solve the structure of unknown crystals, to provide mutants having desirable binding properties, and ultimately, to design, characterize, or identify molecules or chemical entities capable of interfering with the interaction between CD40 and CD40L.

Additional features and advantages of the invention will be set forth in the description which follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and other advantages of the invention will be realized and attained by the compositions and methods particularly pointed out in the written description and claims hereof, as well as in the appended drawings.

To achieve these and other advantages, and in accordance with the purpose of the invention, as embodied and broadly described herein, the invention relates to a crystal of CD40L. More particularly, the invention relates to a crystal formed by a functional fragment of the extracellular domain of sCD40L(116-261), wherein the crystal has cell constants a=b=77.17Å, c= 90.46Å, $\alpha = \beta = 90°$, $\gamma = 120°$, and a space group of R3, and equivalents of that crystal. The claimed crystals of CD40L are substantially described by the structural coordinates identified in Table 1. The claimed crystals in certain embodiments are characterized by a binding site moiety comprising Ile127,

Ser128, Glu129, Ala130, Ser131, Thr135, Ser136, Ala141, Glu142, Lys143, Gly144, Tyr145, Tyr146, Cys178, Asn180, Ser185, Gln186, Ala187, Pro188, Ile190, Ala191, Ser192, Ser197, Pro198, Gly199, Arg200, Phe201, Glu202, Arg203, Ile204, Arg207, Ala209, Thr211, Pro217, Cys218, Gly219, Gln220, Glu230, Leu231, Gln232, Asn240, Val241, Thr242, Asp243, Ser245, Val247, Ser248, His249, Gly250, Thr251, Gly252 and Phe253.

Additionally, the invention relates to crystals of molecules having a binding site comprising at least Arg207, and preferably, amino acids Lys143, Arg203, Arg207 and Tyr145. The claimed crystals in certain embodiments, are formed from molecules having a binding site moiety comprising Arg207 surrounded by at least two hydrophobic residues. Mutants, homologs, co-complexes and fragments of the claimed crystals are also contemplated herein.

The claimed invention in certain embodiments relates to heavy atom derivatives of the crystallized form of a sCD40L(116-261), and, more specifically, the heavy atom derivatives of the crystallized form of CD40L described above. In various embodiments, the claimed invention relates to methods of preparing crystalline forms of CD40L, or fragments thereof, by providing an aqueous solution comprising at least a fragment of CD40L, providing a reservoir solution comprising a precipitating agent, mixing a volume of the CD40L solution with a volume of the reservoir solution and crystallizing the resultant mixed volume. In certain embodiments, the crystal is derived from an aqueous solution comprising sCD40L(116-261). In various embodiments, the concentration of CD40L in the aqueous solution is about 1 to about 50 mg/ml, preferably about 5 mg/ml to about 15 mg/ml, and most preferably, about 10 mg/ml. The precipitating agents used in the invention may be any precipitating agent known in the art, preferably one selected from the group consisting of sodium citrate,

ammonium sulfate and polyethylene glycol. Any concentration of precipitating agent may be used in the reservoir solution, however it is preferred that the concentration be about 1.0 M to about 1.5 M, more preferably about 1.2 M. Similarly, the pH of the reservoir solution may be varied, preferably between about 4 to about 10, most preferably about 7.5.

Various methods of crystallization can be used in the claimed invention, including, but not limited to, vapor diffusion, batch, liquid bridge, or dialysis. Vapor diffusion crystallization is preferred.

Additionally, the claimed invention relates to methods of using the claimed crystal, and the structure coordinates in methods for screening, designing, or optimizing molecules or other chemical entities that may interfere with the interaction between CD40 and CD40L. Thus, the structural coordinates of CD40L or portions thereof can be used to solve the crystal structure of a mutant, homologue or co-complex of CD40L or a fragment thereof, as well as to solve other unknown crystals which asociate with CD40L or fragments thereof.

In some embodiments, the structural coordinates of the CD40L can be used to evaluate a chemical entity to obtain information about the binding of the chemical entity to CD40L. The claimed invention also relates to the structural coordinates described in Table 1. The structural coordinates can be used to characterize chemical entities which interfere with the relationship between CD40 or CD40L such as inhibitors or agonists. The coordinates can also be used to optimize binding characteristics, to determine the orientation of ligands in the binding site of CD40L or CD40. One skilled in the art will appreciate the numerous uses of the claimed invention in the areas of drug design, screening and optimization of drug candidates, as well as in determining additional unknown crystal structures.

In various embodiments, the claimed invention relates to a machine readable data storage medium having a data storage material encoded with machine readable data, which, when read by an appropriate machine, can display a three dimensional representation of a crystal. The crystals displayed comprise a fragment of CD40L such as that described by the coordinates in Table 1, or a crystal having a binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

In other embodiments, the claimed invention relates to a method for determining a at least a portion of a three dimensional structure of a chemical entity or molecular complex by calculating phases from the structural coordinates of a crystal of a fragment of CD40 ligand, calculating the electron density map from the phases obtained, and then determining at least a portion of the unknown structure based upon the electron density map.

In yet other embodiments, the invention relates to methods for evaluating the ability of a chemical entity to associate with CD40 or CD40L. The methods employ computational or experimental means to perform a fitting operation between the chemical entity and the CD40 or CD40L to obtain data related to the association, and analyzing the data to determine the characteristics. Chemical entities identified by these methods which are capable of interfering with the in vivo or in vitro association between CD40 and CD40 L are also encompassed by the claimed invention. The claimed chemical entities may comprise binding sites substantially similar to those of CD40L, or, alternatively may comprise binding sites capable of associating with the binding sites of CD40L.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed.

The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention, and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a depiction of the representative regions of a $2F_o$-F_c electron density map contoured at 2.5σ showing residues in the vicinity of the CD40 binding site.

Figure 2 is an electron density map showing a view of a network of 3 tyrosine and 3 histidine residues formed in the vicinity of the center of the trimer of CD40.

Figure 3 is a stereo drawing of the 3-dimensional structure of human CD40L specifically, CD40L Cα backbone.

Figure 4 is a ribbon representation and secondary structure assignment of CD40L.

Figure 5 is a sequence alignment of the TNF-like domains of TNFα, Lτα and CD40L based on structural considerations.

Figure 6 is a graph of temperature factors of main chain atoms as a function of residue number. Residues are numbered with Pro120 as residue 1.

Figure 7 is a depiction of residues involved in Hyper-IgM and designed mutations. The third monomer has been removed for clarity.

Figure 8 is a summary of sCD40L(116-261) crystallographic and refinement data.

DETAILED DESCRIPTION OF THE INVENTION

In order that the invention described herein may be more fully understood, the following detailed description is set forth.

The present invention relates to a crystal of a soluble fragment of the extracellular domain of the CD40 ligand. Specifically, it relates to a crystal of a protein comprising the sequence from Gly116 to Leu261 (sCD40L(116-261)), the structure of sCD40L(116-261) as determined by X-ray crystallography, and the use of the sCD40L(116-261) structure and that of its homologs, mutants and co-complexes to design, identify, characterize, screen and/or optimize candidate inhibitors or agonists of CD40L activity.

A. DEFINITIONS

The term CD40 ligand ("CD40L") as used herein refers to a genus of polypeptides which are capable of binding to CD40, or homologs or fragments thereof. The term as used herein includes sCD40L(116-261), homologs, mutants, equivalents and fragments thereof.

The term "co-complex" refers to a CD40L or a mutant or homolog of CD40L in covalent or non-covalent association with a chemical entity.

The term "homolog" refers to a protein having at least about 50% amino acid sequence identity with the protein to which it is being compared.

The term "mutant" refers to a CD40L or fragment thereof, characterized by the replacement, deletion, or insertion of at least one amino acid from the wild-type. Such a mutant may be prepared, for example, by expression of a CD40L previously altered in its coding sequence by oligonucleotide-directed mutagenesis.

The term " positively charged amino acid" includes any amino acid, natural or unnatural, having a positively charged side chain under normal physiological conditions.

Examples of positively charged naturally occurring amino acids are arginine, lysine and histidine.

The term "negatively charged amino acid" includes any amino acid, natural or unnatural, having a negatively charged side chain under normal physiological conditions. Examples of negatively charged naturally occurring amino acids are aspartic acid and glutamic acid.

The term "hydrophobic amino acid" means any amino acid having an uncharged, nonpolar side chain that is relatively insoluble in water. Examples are alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophane and methionine.

The term "hydrophilic amino acid" means any amino acid having an uncharged, polar side chain that is relatively soluble in water. Examples are serine, threonine, tyrosine, asparagine, glutamine, and cysteine.

The term "altered surface charge" means a change in one or more of the charge units of a mutant polypeptide, at physiological pH, as compared to CD40 ligand. The change in surface charge can be determined by measuring the isoelectric point (pI) of the polypeptide molecule containing the substituted amino acid and comparing it to the pI of the wild-type molecule.

The term "associating with" refers to a condition of proximity between two chemical entities, or portions thereof, for example, a CD40 ligand or portions thereof and a chemical entity. The association may be non-covalent, wherein the juxtaposition is energetically favored by hydrogen bonding, van der Waals interaction, or electrostatic interaction, or it may be a covalent association.

The term "binding site" refers to any or all of the sites where a chemical entity binds or associates with another entity.

The term "structural coordinates" refers to the

coordinates derived from mathematical equations related to the patterns obtained on diffraction of a monochromatic beam of X-rays by the atoms (scattering centers) of molecule in crystal form. The diffraction data are used to calculate an electron density map of the repeating units of the crystal. Those skilled in the art will understand that the data obtained are dependent upon the particular system used, and hence, different coordinates may in fact describe the same crystal if such coordinates define substantially the same relationship as those described herein. The electron density maps are used to establish the positions of the individual atoms within the unit cell of the crystal.

Those of skill in the art understand that a set of structural coordinates determined by X-ray crystallography is not without standard error. Table 1 is the atomic coordinates of sCD40L(116-261). For the purpose of this invention, any set of structural coordinates of CD40L(116-261) that have a root mean square deviation of equivalent protein backbone atoms of less than about 2 Å when superimposed--- using backbone atoms-- on the structural coordinates in Table 1 shall be considered identical. Preferably the deviation is less than about 1Å and more preferably less than about 0.5Å.

The term "heavy atom derivatization" refers to a method of producing a chemically modified form of a crystallized CD40 ligand. In practice, a crystal is soaked in a solution containing heavy metal atom salts, or organometallic compounds, e.g., lead chloride, gold thiomalate, thimerosal or uranyl acetate, which can diffuse through the crystal and bind to the surface of the protein. The location of the bound heavy metal atom(s) can be determined by X-ray diffraction analysis of the soaked crystal. This information can be used to generate the phase information used to construct the three-dimensional structure of the molecule.

The term "unit cell" refers to a basic shaped block. The entire volume of a crystal may be constructed by regular assembly of such blocks. Each unit cell comprises a complete representation of the unit of pattern, the repetition of which builds up the crystal.

The term "space group" refers to the arrangement of symmetry elements of a crystal.

The term "molecular replacement" refers to a method that involves generating a preliminary structural model of a crystal whose structural coordinates are unknown, by orienting and positioning a molecule whose structural coordinates are known e.g. the CD40 ligand coordinates in Table 1, within the unit cell of the unknown crystal, so as to best account for the observed diffraction pattern of the unknown crystal. Phases can then be calculated from this model, and combined with the observed amplitudes to give an approximate Fourier synthesis of the structure whose coordinates are unknown. This in turn can be subject to any of the several forms of refinement to provide a final accurate structure of the unknown crystal. (See, e.g., Lattman, E., "Use of the Rotation and Translation Functions", Methods in Enzymology, 115, pp. 55-77 (1985); Rossman, ed., "The Molecular Replacement Method", Int. Sci. Rev. Ser. No. 13, Gordon and Breach, New York (1972). Specifically incorporated by reference herein.) Using the structural coordinates of CD40 ligand provided by this invention, molecular replacement may be used to determine the structural coordinates of a crystalline co-complex, unknown ligand, mutant, homolog, or of a different crystalline form of CD40 ligand. Additionally, the claimed crystal and its coordinates may be used to determine the structural coordinates of a chemical entity which associates with CD40L or CD40.

The term "chemical entity" as used herein shall mean, for example, any molecule, molecular complex, compound or

fragment thereof.

CD40L mutants may be generated by site-specific incorporation of natural or unnatural amino acids into CD40L using general biosynthetic methods known to those skilled in the art. For example, the codon encoding the amino acid of interest in wild-type CD40L may be replaced by a "blank" nonsense codon, such as TAG, using oligonucleotide-directed mutagenesis. A suppressor tRNA directed against this codon can then be chemically aminoacylated in vitro with the desired amino acid. The aminoacylated tRNA can then be added to an in vitro translation system to yield a mutant sCD40L with the site-specific incorporated amino acid.

The term soluble fragment of CD40L and any equivalent term used herein, refers to a functional fragment of CD40L. The term "functional" as used in this context refers to a soluble fragment of the extracellular domain that is capable of binding to, or associating with, a CD40, CD40-Ig fusion protein or any fragments or homologs thereof, including molecular complexes comprising fragments thereof. Such binding may be demonstrated through immunoprecipitation experiments, using standard protocols known in the art.

A. CD40L, its Crystal, and its Biological Implications

It will be understood that throughout the specification and claims, the positional location of the amino acids described is not an absolute value, but rather, defines the relative relationship of the residues. Thus it is intended that the present invention encompass the sequences having the same or similar relative positions.

For the first time, the present invention permits the use of molecular design techniques to design, screen and optimize chemical entities and compounds, including inhibitory compounds, capable of binding to the active site or accessory binding site of CD40L, in whole or in part.

CD40 ligand (CD40L) is a T cell membrane-bound pleiotropic cytokine of considerable biomedical interest because of its involvement in important immune system functions mediated by the binding of CD40L to CD40. CD40 is a molecule expressed on the surface of B cells and other cell types. CD40L is found in various organisms, such as humans, mice, rats, pigs, etc. The claimed invention is not intended to be limited to any particular species or organism.

CD40L is a member of the tumor necrosis factor family of ligands. The crystal structure of another member of this family, lymphotoxin-α (LTα) complexed with its receptor has been described and has be used as a framework for understanding the CD40L crystal structure. However, despite certain similarities, the differences between the LT system and the CD40 system confirm that the two ligand-receptor systems utilize spatially overlapping, but nonidentical and nonconserved sites of contact residues with different molecular determinants of binding.

Considering the complexity and overlap of the various immune system processes, the fact that CD40 signaling appears to be non-redundant suggests that inhibiting CD40L signaling may have important therapeutic applications. The crystal structure of CD40L presented here is expected to be useful in the design, identification, characterization and optimization of such therapeutic agents.

The following detailed description of applicants invention encompasses the (a) crystal structure of CD40L(116-261) and the coordinates thereof, (b) the binding sites of CD40L, (c) methods of making a CD40L crystal, and (d) methods of using the CD40L crystal and its structural coordinates.

(a.) Crystal Structure of CD40 Ligand

The claimed invention provides crystals of CD40 ligand, as well as the structure of CD40 ligand determined therefrom. Specifically, the claimed invention provides

crystals of a fragment of CD40 ligand(116-261) having unit cells which are rhombohedral, and having the following dimensions a=b=77.17 Å and c=90. 46 Å, α=β= 90 Å and γ = 120 Å. Almost all of the residues of CD40 ligand fragment, except for residues 116-119 of the N terminus, are well defined in the final electron density map shown in Figure 1. There are areas of weak density for residues 182-186 and 210-220. The current model consists of 142 amino acid residues and 95 water molecules with a crystallographic R factor of 21.8 % and an R_{free} of 29.1% for data between 7.5 Å and 2 Å. The Ramachandran diagram shows that 140 out of the 142 amino acid residues have (ϕ,ψ) angles within the allowed regions. The exceptions are residue Cys218, which is involved in the formation of a disulfide bridge, and Lys143.

CD40 ligand folds as a sandwich of two β sheets with jellyroll or Greek key topology (Figure 3). The dimensions of the molecule are 25 Å x 30 Å x 50 Å. The overall fold is similar to that of TNF-α and LT-α. The notation used herein is that described in Eck et.al., "The Structure of Human Lymphotoxin", J. Biol. Chem., 267, 2119-2112, for the β strands and other structural features. One β sheet consists of strands A"AHCF, and the other of strands B'BGDE. To assess the degree of structural similarity between TNF-α, LT-α and CD40 ligand, the sequences were aligned to maximize the overlap of equivalent β strand residues. The equivalent Cα atoms from the β strands were then used to superimpose the molecular structures. The root mean square (rms) positional deviation of 86 equivalent Cα atoms of superimposed TNF-α and CD40L molecules is 1.10 Å. In the case of the LT/CD40L pair, the rms deviation for the 100 equivalent Cα atoms is 1.03 Å. The positions of the residues are highly conserved in the core and β strand regions, but differ significantly in certain loops, such as the AA", CD and EF loops. An alignment of the TNF-α, LT-α

and CD40L sequences based on the best structural superimpositions was made. Figure 5. Most residues of the hydrophobic core of CD40L maintain the identity of the character encountered in the equivalent residues of TNF and LT. There are local compensatory, conformational changes to accommodate side-chain changes, such as the substitution of Leu33 of LT-α to Val36 in CD40L, which results in a shift of the side chain of Leu161 to fill the empty space.

Three CD40L molecules form a trimer similar to that observed in the crystal structures of TNF-α and LT-α. The trimer has the shape of a truncated pyramid. The three-fold axis of the trimer is approximately parallel to the β strands of each subunit. The interface between the subunits is formed mainly by two tyrosines, two histidines and one leucine. Thus the "aromatic tiling" observed in LT is not as prevalent in CD40L. Tyr170 and His224 from each monomer form an unusual cluster of two triads along the threefold axis of the trimer.

The crystal packing of CD40L trimers was also studied. Each CD40L monomer forms one crystal contact that involves loops DE (residues 198-201), FG (232-233) and BC (64-166) from one molecule and loops CD (182-186), AA" (131-135) and GH (245-246) from the neighboring molecule. Sixteen water molecules are involved in the formation of the contact.

The CD and EF loops of CD40L at the "top" of the molecule are shorter than those of TNF-α and LT-α. However, despite its shorter length, the CD loop contains 1.5 turns of a helix in a similar manner to the equivalent loop in LT. Analogs to LT regions of poor electron density occur within these loops that may indicate disorder. In addition, a plot of temperature factors (Figure 6) suggests that the CD and EF loops are the most mobile parts of the molecule. The C and F strands are linked by a disulfide bridge between Cys178 and Cys218 that may play a role in stabilizing the top of the molecule. A disulfide bridge is also present in

TNF but it is located at a different position. Although the AA" loop does not differ much between TNF and LT, in CD40L it has a very different structure. In CD40L the AA" loop is shifted away from the CD40L binding site relative to TNF and LT. The unusual conformation of the AA" loop may be related to its extensive participation in the crystal contact. A short 10 helical structure is found within the GH loop, and β bulge occurs in the middle of strand E. The N and C terminii lie close to each other at the base of the trimer. Residues 116-119 of the N terminus of the construct are poorly defined in the electron-density map, therefore, the first well ordered residue is Pro120. Residues 116-119 presumably constitute part of the stalk connecting the globular domain to the transmembrane segment. This 65-residue stalk is unusually long compared with the equivalent segments in other members of the TNF family.

A single glycosylation site is predicted from analysis of the CD40L sequence. Biochemical experiments revealed that residue Asn240 of the CD40L protein used for the crystallization has an N-linked carbohydrate attached to it. A 2F-Fc map shows electron density in the vicinity of the side chain of Asn240 which presumably corresponds to the attached carbohydrate. However, because the density is not well defined, the current model contains no carbohydrate atoms at this position.

(b.) Binding Site

By analogy with the LT-TNFR complex crystal structure, the CD40 binding site consists of a shallow groove formed between two monomers. The activation complex of CD40L-CD40 is expected to be similar to the LT-TNFR complex. On the basis of linkage diagrams showing which residue of LT interacts with which residue of TNFR, stretches of the CD40L sequence that were likely to constitute the binding site of CD40L were inferred.

Applicants have determined that the CD40L binding site

comprises Arg207 in close physical proximity to at least two hydrophobic residues. More specifically, the binding sit comprises at least amino acids Lys143, Arg203, Arg207 and Tyr145. Numerous other residues may be included in the binding site, including, but not limited to, Ile127, Ser128, Glu129, Ala130, Ser131, Thr135, Ser136, Ala141, Glu142, Lys143, Gly144, Tyr145, Tyr146, Cys178, Asn180, Ser185, Gln186, Ala187, Pro188, Ile190, Ala191, Ser192, Ser197, Pro198, Gly199, Arg200, Phe201, Glu202, Arg203, Ile204, Arg207, Ala209, Thr211, Pro217, Cys218, Gly219, Gln220, Glu230, Leu231, Gln232, Asn240, Val241, Thr242, Asp243, Ser245, Val247, Ser248, His249, Gly250, Thr251, Gly252 and Phe253.

One skilled in the art will appreciate that modifications to the binding site, i.e. substitutions, insertions or deletions, may be made and the binding site will maintain its activity. Thus, it is contemplated that a binding site comprising at least 50 % homology to that defined above is encompassed by the invention. More specifically, it is preferred to have about 60% homology, and most preferred about 75-99% homology. Variations in the percentage of homology to the binding site defined above may alter the binding properties of the resultant entity. In a preferred embodiment, the binding site comprises at least 30 residues from the list defined above.

The binding site was expected to contain residues mostly from the AA" and DE loops. In addition, residues from the CD and GH loops and B strands, C, D, G and H were expected to be involved in CD40 binding. Virtually no sequence conservation exists between CD40L and LT-α or TNF-α in these regions. Although Ser131 of the AA" loop appears to be conserved in the sequence alignment, the structural superimposition shows that it is relatively distant from the equivalent position (Ser38) in LT. The Tyr45 side chain of the AA" loop of CD40L is disordered, but is found in an

equivalent position at Arg51 of LT, and, like Arg51 in the LT-α-TNFR complex, may have an extended and well-defined conformation in the complex. On the DE loop, only Phe201 is conserved (equivalent to Phe110 of LT). Phe201 adopts a similar conformation to that of Phe110 in the LT-α-TNFR complex, whereas in the uncoupled LT, Phe110 adopts a different conformation. The Arg200 side chain in the DE loop is involved in a crystal contact. The conformation of loops DE AA" and GH is likely to be affected by the crystal contact, thus, it is expected that their structure will be different in a complex. The conformational changes associated with complex formation in the case of LT, although minor, may be indicative of the magnitude of changes that occur upon CD40 binding to CD40L.

A mixture of both hydrophobic and hydrophilic residues form the surface of the binding site. The LT-TNFR binding interface can be considered as separate upper and lower regions. In a similar manner to LT, the upper region in CD40L has more hydrophobic and non-charged polar residues than the lower region. Arg207 is an important residue, lying in the area where the upper and lower regions connect. The Arg207 side chain adopts an extended conformation pointing towards the putative location of the CD40 receptor, and is surrounded by at least two surface-exposed hydrophobic residues and a serine residue (Ser192). Two of the hydrophobic residues (190 and Phe253) belong to the neighboring subunit, whereas Ile204 is from the same subunit. Murine CD40L has a lysine in the position equivalent to that of Arg207 of human CD40L, and this lysine also seems likely to be surrounded by hydrophobic residues. The hydrophobic environment of the arginine may amplify the strength of a possible electrostatic interaction between this residue and an acidic residue of CD40.

(c.) Methods of Making a CD40L Crystal

In various embodiments, the claimed invention relates

to methods of preparing crystalline forms of CD40L, or fragments thereof by first providing an aqueous solution comprising CD40L or a fragment of CD40L. A reservoir solution comprising a precipitating agent is then mixed with a volume of the CD40L solution and the resultant mixed volume is then crystallized. In certain embodiments, the crystal is derived from an aqueous solution comprising sCD40L(116-261). The concentration of CD40L in the aqueous solution may vary, and is preferably about 1 to about 50 mg/ml, more preferably about 5 mg/ml to about 15 mg/ml, and most preferably, about 10 mg/ml. Similarly, precipitating agents used in the invention may vary, and may be selected from any precipitating agent known in the art. Preferably the precipitating agent is selected from the group consisting of sodium citrate, ammonium sulfate and polyethylene glycol. Any concentration of precipitating agent may be used in the reservoir solution, however it is preferred that the concentration be about 1.0 M to about 1.5 M, more preferably about 1.2 M. The pH of the reservoir solution may also be varied, preferably between about 4 to about 10, most preferably about 7.5. One skilled in the art will understand that each of these parameters can be varied without undue experimentation and acceptable crystals will still be obtained. In practice, once the appropriate precipitating agents, buffers or other experimental variables are determined for any given growth method, any of these methods or any other methods can be used to grow the claimed crystals. One skilled in the art can determine the variables depending upon his particular needs.

Various methods of crystallization can be used in the claimed invention, including, but not limited to, vapor diffusion, batch, liquid bridge, or dialysis. Vapor diffusion crystallization is preferred. See, e.g. McPherson et al., "Preparation and Analysis of Protein Crystals", Glick,. Ed., pp 82-159, John Wiley & Co. (1982); Jancarik

et.al., "Sparse matrix sampling: a screening method for crystallization of protein", J. Appl. Cryst. 24, 409-411 (1991), specifically incorporated by reference herein.

In vapor diffusion crystallization, a small volume (i.e. a few milliliters) of protein solution is mixed with a solution containing a precipitating agent. This mixed volume is suspended over a well containing a small amount, i.e. about 1 ml, of precipitating solution. Vapor diffusion from the drop to the well will result in crystal formation in the drop.

The dialysis method of crystallization utilizes a semipermeable size exclusion membrane which retains the protein but allows small molecules (i.e. buffers and precipitating agents) to diffuse in and out. In dialysis, rather than concentrating the protein and the precipitating agent by evaporation, the precipitating agent is allowed to slowly diffuse through the membrane and reduce the solubility of the protein while keeping the protein concentration fixed.

The batch methods generally involve the slow addition of a precipitating agent to an aqueous solution of protein until the solution just becomes turbid, at this point the container can be sealed and left undisturbed for a period of time until crystallization occurs.

Thus, applicants intend that the claimed invention encompass any and all methods of crystallization. One skilled in the art can choose any of such methods and vary the parameters such that the chosen method results in the desired crystals.

(d.) Use of CD40L Crystal and its Coordinates

The claimed crystals, and coordinates describing them, permit the use of molecular design techniques to design, select and synthesize chemical entities and compounds, including inhibitory compounds or agonists capable of binding to, or associating with, the binding site of CD40L

or CD40 in whole or in part.

One approach enabled by this invention is the use of the structural coordinates of CD40L to design chemical entities that bind to or associate with, CD40L and alter the physical properties of the compounds in different ways. Thus, properties such as, for example, solubility, affinity, specificity, potency, on/off rates or other binding characteristics may all be altered and/or optimized.

One may design desired chemical entities by probing a CD40L crystal with a library of different entities to determine optimal sites for interaction between candidate chemical entities and CD40L. For example, high resolution x-ray diffraction data collected from crystals saturated with solvent allows the determination of where each type of solvent molecule sticks. Small molecules that bind tightly to those sites can then be designed and synthesized and tested for the desired activity. Once the desired activity is obtained, the molecules can be further optimized.

The claimed invention also makes it possible to computationally screen small molecule data bases or computationally design chemical entities or compounds that can bind in whole, or in part, to CD40 or CD40L. They may also be used to solve the crystal structure of mutants, co-complexes, or of the crystalline form of any other molecule homologous to, or capable of associating with, at least a portion of CD40L.

One method that may be employed for this purpose is molecular replacement. An unknown crystal structure, which may be any unknown structure, such as, for example, another crystal form of CD40L, a CD40L mutant, or a co-complex with CD40, or any other unknown crystal of a chemical entity which associates with CD40L which is of interest, may be determined using the structural coordinates of this invention, set forth in Table 1. Co-complexes with CD40L may include, but are not limited to, CD40-CD40L, CD40L-small

molecule, and CD40-derived fragments. This method will provide an accurate structural form for the unknown crystal more quickly and efficiently than attempting to determine such information without the claimed invention.

The information obtained can thus be used to optimize potential inhibitors or agonists of CD40L,or CD40, and more importantly, to design and synthesize novel classes of chemical entities which will affect the relationship between CD40L and CD40.

The design of compounds that inhibit or agonize CD40L according to this invention generally involves consideration of at least two factors. First, the compound must be capable of physically or structurally associating with CD40L or CD40. The association may be any physical, structural, or chemical association, such as, for example, covalent or noncovalent bonding, van der Waals interactions, hydrophobic or electrostatic interactions.

Second, the compound must be able to assume a conformation that allows it to associate with CD40 or CD40L. Although not all portions of the compound will necessarily participate in the association with CD40 or CD40L, those non-participating portions may still influence the overall conformation of the molecule. This in turn may have a significant impact on the desirability of the compound. Such conformational requirements include the overall three-dimensional structure and orientation of the chemical entity or compound in relation to all or a portion of the binding site.

The potential inhibitory or binding effect of a chemical compound on CD40 or CD40L may be analyzed prior to its actual synthesis and testing by the use of computer modeling techniques. If the theoretical structure of the given compound suggests insufficient interaction and association between it and CD40 or CD40L, the need for synthesis and testing of the compound is obviated. However,

if computer modeling indicates a strong interaction, the molecule may then be synthesized and tested for its ability to bind to CD40 or CD40L. Thus, expensive and time consuming synthesis of inoperative compounds may be avoided.

An inhibitory or other binding compound of CD40 or CD40L may be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with the individual binding sites of CD40L.

Thus, one skilled in the art may use one of several methods to screen chemical entities or fragments for their ability to associate with CD40L and more particularly, with the individual binding sites of sCD40L(116-261). This process may begin by visual inspection of, for example, the binding site on a computer screen based on the CD40L coordinates in Table 1. Selected fragments or chemical entities may then be positioned in a variety of orientations, or "docked", within an individual binding pocket of CD40L. Docking may be accomplished using software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER.

Specialized computer programs may be of use for selecting interesting fragments or chemical entities. (GRID, available from Oxford University, Oxford, UK; MCSS or CATALYST, available from Molecular Simulations, Burlington, MA; AUTODOCK, available from Scripps Research Institute, La Jolla, CA; DOCK available from University of California, San Francisco, CA., XSITE, University College of London, UK.)

Once suitable chemical entities or fragments have been selected, they can be assembled into an inhibitor or agonist. Assembly may be by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen, in

relation to the structural coordinates disclosed herein.

Alternatively, one may design the desired chemical entities "de novo", experimentally, using either an empty binding site, or optionally including a portion of a molecule with desired activity. Thus, for example, one may use solid phase screening techniques where either CD40L or a fragment thereof, or candidate chemical entities to be evaluated are attached to a solid phase thereby identifying potential binders for further study or optimization.

Basically, any molecular modeling techniques may be employed in accordance with the invention; these techniques are known, or readily available to those skilled in the art. It will be understood that the methods and compositions disclosed herein can be used to identify, design or characterize not only entities which will associate or bind to CD40L, but alternatively to identify, design or characterize entities which, like CD40L, will bind to CD40 thereby disrupting the CD40L-CD40 interaction. The claimed invention is intended to encompass these methods and compositions broadly.

Once a compound has been designed or selected by the above methods, the efficiency with which that compound may bind to CD40L may be tested and optimized using computational or experimental evaluation. Various parameters can be optimized depending on the desired result. These include, but are not limited to, specificity, affinity, on/off rates, hydrophobicity, solubility and other characteristics readily identifiable by the skilled artisan.

Thus, one may optionally make substitutions, deletions, or insertions in some of the components of the chemical entities in order to improve or modify the binding properties. Generally, initial substitutions are conservative, i.e the replacement group will have approximately the same size, shape, hydrophobicity and

charge as the original component.

The present invention also enables the design of mutants of CD40L and the solving of their crystal structure. More particularly, the claimed invention enables one skilled in the art to determine the location of binding sites and interfaces, thereby identifying desirable sites for mutation.

For example, mutation may be directed to a particular site or combination of sites on the CD40L, by replacing or substituting one or more amino acid residues. Such mutants may have altered binding properties which may or may not be desirable.

The mutants may be prepared by any methods known in the art, such as for example, site directed mutagenesis, deletion or addition, and then tested for any properties of interest. For example, mutants may be screened for an altered charge at a particular pH, tighter binding, better specificity etc.

The CD40L structure suggests that most, if not all, of the naturally occurring single-site HIGMS mutations affect the folding and stability of the protein rather than the binding site directly. Indeed, more severe HIGMS mutations involving deletions or other drastic changes would be expected to perturb the structure much more than single-site mutations. In addition, only two of the six binding-site residues selected for mutagenesis were found to be critical for CD40 binding.

A similar low frequency of hits was found in an analogous mutational study of LTα. Van Ostade et al., "Structure activity studies of human tumour necrosis factors.", Protein Engineering 7: 5-22 (1994). This observation raises the question of whether mutations of individual CD40L binding-site residues are generally sufficient to completely disrupt CD40L-CD40 binding. If insufficient, CD40-mediated cellular signaling will continue

and the mutation will be clinically undetectable. This behavior is consistent with the fact that, in the LTα-receptor complex structure, as many as 38 LTα residues are involved in the binding interface. The interface is quite extensive (520 $Å^2$) and is presumably of similar dimensions in the CD40L-CD40 complex. It is expected, therefore, that each residue only contributes a small fraction of the binding energy. However, the interaction of the human growth hormone with its receptor has also been shown to involve a large surface area, yet relatively few key residues contribute most of the binding energy.

Additionally, the claimed invention is useful for the optimization of potential small molecule drug candidates. Thus, the claimed crystal structures can be also be used to obtain information about the crystal structures of complexes of the CD40 ligand and small molecule inhibitors. For example, if the small molecule inhibitor is co-crystallized with CD40 ligand, then the crystal structure of the complex can be solved by molecular replacement using the known coordinates of CD40 ligand for the calculation of phases. Such information is useful, for example, for determining the nature of the interaction between the CD40 ligand and the small molecule inhibitor, and thus, may suggest modifications which would improve binding characteristics such as affinity, specificity and kinetics.

The detrimental effects of several HIGMS mutations on CD40L appear to be caused by destabilizing its structure. For example, Trp140 is a large hydrophobic residue buried inside the protein and removal of its side chain in the HIGMS mutations Trp140→Gly and Trp140→Arg will obviously lead to destabilization of the structure. The valine affected by the Val126→Ala mutation also participates in forming the hydrophobic core. Leu155 is not completely buried but lies in the middle of β strand B. Introduction of proline at this position, as in the Leu155→Pro HIGMS

mutation, may disrupt the formation of the strand. The behavior of the HIGMS mutation Ala235→Pro can be similarly explained. Substitution of Gly144 in the HIGMS mutation Gly144→Glu may result in loss of the conformational freedom necessary at that position to form the corner of the AA" loop. Consequently, the positioning of the adjacent residues, Lys143 and Tyr145, which are known to be involved in binding, is disrupted. An interesting observation is that all of the above mentioned residues appear to be clusters: Trp140, Leu155 and Val126 participate in the formation of an area of the hydrophobic core partially formed by strands A, A", B' and B. Lys143, Gly144 and Tyr145 are exposed surface residues of the AA" loop lying very close to the above mentioned hydrophobic residues. This suggests that the CD40-binding capacity is very sensitive to perturbations in this area of CD40L.

HIGMS mutations Ala123→Glu, Gly227→Val and Thr211→Asp affect residues that are involved in formation of the subunit interface. Ala123 lies near the bottom of the trimer and is within van der Waals distance of three hydrophobic residues (Leu168, Val228 and Leu261) of the neighboring subunit. Gly227 packs against Tyr172 of the neighboring subunit and Thr211 lies on the EF loop close to the intersubunit interface where it appears to interact with Arg181 from the neighboring subunit. Mutations that introduce larger side chains at these positions (e.g. Thr211→Asp) would be expected to disturb the intersubunit packing and possibly prevent trimeritation. Thus, it appears that most of the single-site HIGMS mutations affect the folding and stability of CD40L rather than playing a direct role in CD40 binding. In addition, mutations of only two of the nine targeted surface residues appear to affect CD40 binding.

Mutagenesis studies on CD40L have shown that residues Tyr145 and Lsy143 are directly involved in CD40 binding.

The crystal structure shows that both residues lie on the AA" loop and are surface-exposed. Figure 7. The equivalent loop in the LT-TNFR crystal structure is involved in several ligand receptor contacts suggesting similarities in binding. Tyr145 lies at the corner of the loop and its side chain has no visible electron density which suggests that it is disordered. Atom N1 of Lys143 forms a hydrogen bond with Oδ1 of Glu129. Figure 2. The electron density map also shows that the side chain of Lys143 has an additional conformation. Ser128 and Glu129, two residues involved in the HIGMS mutation Ser128→ArgGlu129→Gly lie close to Lys143 and are well defined in the map. Ser128 is almost completely buried, and its side-chain hydroxyl group is within hydrogen bonding distance of His249. It has been shown that the Glu129-Gly substitute found in the HIGMS mutation affects a solvent accessible residue that might participate in binding to CD40. Bajorath et al., "Identification of Residues on CD40 and its Ligand which are Critical for the Receptor-ligand interaction", Biochemistry 34, 1833-1844 (1995), specifically incorporated herein by reference. One possible candidate for it is the exposed residue Lys143, and loss of its hydrogen bond to Glu129 may destabilize its conformation resulting in reduced CD40 binding. All of these residues are conserved in the murine CD40L sequence.

Five additional single-site mutations, in which residues Ser131, Asn180, Phe201, Glu202 and Asn240 (located at the binding site) were substituted by alanine, established that these residues are not critical for CD40 binding. In addition, two other mutations at residue positions outside of the binding site (Thr135 and Asp243) were also shown not to affect binding. According to the structure, Ser131 and Thr135 lie on the AA" loop and their side chains are not exposed. Asn240, the site of glycosylation, is exposed to the solvent. Because the

attached sugar would be expected to make Asn240 unavailable for interaction with CD40, we conclude that it is probably not involved in CD40 binding, in agreement with the mutagenesis results. Asp243 is a semi-exposed residue and it makes a hydrogen bond to Asn186. Asn180 is an exposed residue at the top of the molecule. Its side chain makes hydrogen bonds to residues 183 and 216. Phe201 and Glu202 both lie on the DE loop in the binding site area and are exposed to the solvent.

C. EXAMPLES

1. Determination of Crystal Structure of sCD40L(116-261)

A. Crystallization

Buffer chemicals were purchased from Fisher (Boston, MA). Crystallization condition screenings were done with the Crystal Screen™ kit from Hampton Research (Riverside, CA). A soluble fragment of the extracellular domain of human CD40 ligand containing amino acid residues Gly116 to the C-terminal residue Leu261 was produced in soluble form and purified as follows:

The gene encoding the sCD40L(116-261) sequence of amino acids G116-L261 was cloned into the *Pichia pastoris* expression vector pWS106 and the sCD40L(116-261) protein was expressed by using standard protocols. Peitsch et al., "A B-D Model for the CD40 Ligand Predicts That it is a Compact Trimer Similar to the Tumor Necrosis Factors.", Int. Immunol. 5, 233-238. (1993). pWS106 is a variant of vector pPIC9 (Invitrogen) with the NcoI site at 3634 nt in the 3'-AOX1 flanking region deleted by site directed mutagenesis. The cells were lysed and the medium was dialyzed overnight with 20mM Tris-HCl, pH 6.8 and loaded onto an SP (Pharmacia) column. The bound sCD40L was eluted by 2xPBS, pH 7.2 and buffer-exchanged to 1xPBS.

The protein stock was made available as a solution of 8 mg/ml sCD40L in PBS (20.44 g/lt Na_2HPO_4, 7.73 g/lt Na_2HPO_4-H_2O, 87.66 g/lt NaCl) buffer. Crystals were grown by the vapor diffusion method. (see Jancarik et.al.). In order to find conditions of crystallization, an incomplete factorial screen was set up. In a typical experiment, protein solution was mixed with an equal volume of reservoir solution and a drop of the mixture was suspended under a glass cover slip over the reservoir solution. Crystals were grown out of 1.4 M Na Citrate, 50 mM Na, Hepes pH 7.5 reservoir solution. The crystals are shaped as cubes, are

easy to reproduce and can reach maximum dimensions of almost 1mm on each side (optimal concentration of precipitant to yield the biggest crystals is 1.2 M Na Citrate). Variation of pH between 7 and 8 did not affect crystal quality. Macroseeding techniques were also successful, although not necessary, to get crystals of sufficient quality for data collection. Crystal composition was assayed after washing, dissolution in water and SDS electrophoresis. The gel showed a strong band of about 20,000 daltons and a much fainter band of 17,000 daltons. The fainter band corresponds to non-glycosylated protein while the stronger band corresponds to the glycosylated form.

Those of skill in the art will appreciate that the aforesaid crystallization conditions can be varied. By varying the crystallization conditions, other crystal forms of sCD40L may be obtained. Such variations may be used alone or in combination, and include: varying final protein concentrations between 5 mg/ml and 35 mg/ml; varying the SCD40L to precipitant ratio; varying citrate concentrations between 1.2 M and 2.0 nM; varying pH ranges between 5.5 and 9.5; varying HEPES concentrations between 5 and 395 mM; varying the concentration or type of detergent; varying the temperature between -5 °C and 30 °C; and crystallizing SCD40L by batch, liquid bridge, or dialysis method using the above conditions or variations thereof. See McPherson, A. (1982). Preparation and Analysis of Protein Crystals. (Glick, ed.) pp. 82-159, John Wiley & Co., N.Y., specifically incorporated by reference herein.

B. Data collection and processing

Crystals were mounted inside capillary tubes and tested for diffraction capacity on the X-ray beam of an Elliot GX-13 generator. Oscillation photographs showed strong diffraction to high resolution. Initial inspection of the photographs suggested an almost cubic lattice.

A large crystal (0.8 x 0.8 x 0.8 mm) was equilibrated

gradually in a cryoprotectant solution of 20% glycerol, 1.2 M Na Citrate , 50 mM Na Hepes pH 7.5, mounted on a loop and immediately frozen in a -150 °C liquid nitrogen gas stream. The technique of freezing the crystals essentially immortalizes them and produced a much higher quality data set. A native X-ray data set up to 1.75 Å resolution was collected by using a Nicolet/Siemens multiwire area detector (Siemens, Inc.). The data were integrated and reduced using BUDDHA (25) and the CCP4 program package (The SERC (UK) Collaborative Computing Project No 4, Daresbury Laboratory, UK 1979). The data collection required about 5 days.

Data processing suggested a rhombohedral unit cell with approximate cell dimensions a=b=c=55 Å and $\alpha=\beta=\gamma=91$. To assist calculations, a hexagonal unit cell is defined instead with dimensions a=b=77.17 Å, c=90.46 Å, $\gamma=120$ degrees. Merging of data suggested that the space group is R3. If space group R3 is assumed, then R_{merge} is 6.7%. Assuming space group R32, R_{merge} is 16.6%. Table 1 contains information for the data set obtained.

Calculation of the Matthews volume gives VM= 533,610 $A^3/Z*MW=2.97$ assuming Z=9 (#of asymmetric units in unit cell) and MW=20,000 daltons. Eck et.al., J. Biol.Chem. 267, 2119-2112 (1992), specifically incorporated herein by reference. Thus, it was not clear initially whether there were one or two monomers in the asymmetric unit.

C. Molecular replacement

All subsequent molecular replacement and refinement computing was done with the XPLOR program package. Molecular graphics manipulations were done with QUANTA (Molecular Simulations, Inc.) software. Both XPLOR and QUANTA were run on a Silicon Graphics Indigo2 computer workstation.

To investigate whether non-crystallographic symmetry was present, the self rotation function was calculated with 8-4 Å data. A very strong peak was found for $\phi=90°$, $\psi=90°$,

κ=180°, which corresponds to the crystallographic 3-fold axis. This coincides with the 3-fold axis of the trimer of SCD40L. Also, less strong peaks appeared for φ=0°, ψ=30°, κ=180° and φ=0°, ψ=90°, κ=180° and φ=0°, ψ=150°, κ=180° corresponding to 2-fold axes perpendicular to the 3-fold. None of these axes corresponded to non-crystallographic symmetry.

A 3-dimensional model of the human sCD40L was constructed by using the murine CD40L model as a framework, using QUANTA protein homology modeling software. The model was used as a probe for molecular replacement calculations.

Calculation of the cross rotation function by using a 2.5° angle grid and 8-4 Å data produced a strong peak 4.5σ above the mean, at Euler angles θ_1=234.5°, θ_2=5.0°, θ_3=234.5°. Rotation of the model according to that solution and subsequent generation of symmetry-related molecules corresponding to the 3-fold crystallographic symmetry produces a trimer with the 3-fold axis parallel to the z axis, as expected. The exact rotation solution was found with Patterson correlation refinement. No significant second peak was observed in the rotation function.

In order to conduct the translation search, the model probe was rotated according to the first peak of the rotation search and translated so that the center of mass of the trimer would lie on the z axis. This was assumed to be close to the expected position since it appeared that the 3-fold axis of the trimer lay on the z axis of the unit cell. Initial attempts to find a clear peak in the translation function on the xy plane failed. Trimmed model probes, which contained mostly core residues whose structure was more likely to be conserved among different members of the TNF family, produced translation functions with a clustering of peaks at locations close to the expected ones. One of those trimmed models containing all backbone atoms as well as side chain atoms for residues 8-12, 53-62, 90-93,

110-114 and 141-146 produced a peak with a correlation coefficient of 3.3σ above the mean at x=0.053, y=0.053. This peak was the highest on the list of peaks and was close to the expected position.

By generating symmetry related molecules and displaying them with computer graphics it was found that they packed satisfactorily in the unit cell and that there was not enough space for a second molecule. Thus, we concluded that there was only one molecule in the asymmetric unit. The existence of 2-fold symmetry in the self rotation function apparently is a consequence of internal symmetry within the molecule, possibly related to the high content of parallel strands. In fact, the self rotation function calculated from structure factors derived from the model shows peaks corresponding to 2-fold symmetry.

D. Model building and crystallographic refinement

The param19.pro stereochemical parameter set of XPLOR package was used for all refinement calculations. The partial model that was used to find the solution of the translation function was subjected to rigid body refinement by using 7.5-2.5 Å data. The R and R_{free} (29) factors after the initial rigid body refinement were 49.9% and 51.4% respectively. The test data set used for the calculation of R_{free} contained 10% of the data. The partial model was subjected to 40 steps of conventional positional refinement and a cycle of simulated annealing with an initial temperature of T=2500 K. The R and R_{free} factors dropped to 31.2% and 43.1% respectively. To reduce model bias, a partial model was used for map calculation and refinement. The resolution range used was 7.5-2.5 Å. Simulated annealing omit maps (30) calculated by consequently omitting 10% of the model each time showed which parts of the model could be used for phasing. Thus, the model was modified to include only residues sufficiently well defined in annealed omit maps. The initial model included 815 atoms out of a

total of 1374 atoms of the complete model. $3F_o - 2F_c$ maps were used for cycles of model building and refinement. Typically, cycles consisted of model building, positional refinement and B-factor refinement. Occasionally simulated annealing was performed. As the phases improved, more atoms were added into the model. Initially, grouped B-factors were assigned for each strand (one for main chain and the one for side chain atoms). Later, grouped B-factors and finally individual atomic B-factors where refined for each residue. Only manual structure modifications that resulted in lower R_{free} after refinement were accepted. When R and R_{free} reached 31.1% and 36.4% respectively, the resolution was extended to 2.25 Å and finally to 2 Å. Water molecules were added by using the X-solvate utility of QUANTA 4.1. Both occupancies and temperature factors were refined for the water molecules. Figure 8 summarizes information regarding crystallographic data and refinement. Table 1 lists the atomic coordinates of sCD40L(116-261).

The coordinates of the crystal structure of a sCD40L may be used in the structure-based design of small molecule inhibitors of CD40L, computational drug design and iterative structure optimization.

a. Computational drug design

Small molecule inhibitors can be designed using computational approaches. These approaches are also known as de novo drug design. In brief, the crystal structure coordinates of the sCD40L are the input for a computer program, such as DOCK. Programs such as DOCK output a list of small molecule structures that are expected to bind to CD40L. These molecules can then be screened by biochemical assays for CD40L binding.

Typically, biochemical assays that screen molecules for their ability to bind to CD40L are competition-type assays. In such assays, the molecule is added to the assay solution and the degree of inhibition is

measured using conventional methodology.

b. Iterative cycles of structure optimization

The crystal structures of complexes formed between sCD40L and small molecule inhibitors may be solved. In brief, small molecule inhibitors are typically found using the crystal structure coordinates of a sCD40L either by the computational approaches mentioned above or by the screening of small molecule libraries. The small molecule inhibitor is then co-crystallized with sCD40L and the crystal structure of the complex is solved by molecular replacement. Molecular replacement requires the coordinates of a sCD40L for the calculation of phases. The information collected from these experiments can be used to optimize the structure of small molecule inhibitors by clarifying how small molecules interact with the protein target. This suggests ways of modifying the small molecule to improve its physicochemical properties, such as affinity, specificity, and kinetics with regard to the CD40L target.

In addition to being necessary for computational drug design and structure optimization, the crystal coordinates of sCD40L are useful for analyzing the CD40L binding site. Through such analysis, it was determined that a particularly attractive region for drug targeting is in the vicinity of Arg207. Arg207 is situated in a region surrounded by three hydrophobic residues and one moderately hydrophobic residue. This grouping appears to demark one wall of a binding groove formed between two CD40L monomers. While not wishing to be bound by theory, it appears that Asp84, or Glu117 of CD40 probably interacts with CD40L at this site. The hydrophobic residues that surround Arg207 may result in increased strength of electrostatic interactions between Arg207, Asp84 and Glu117 of CD40. Specifically, it appears that Glu117 and Asp84 are important. The above observations and hypotheses suggest that this region may contribute significantly to the binding

energy of CD40L/CD40 interactions, and therefore, is an attractive target for inhibitor design. Site mutations studies can be used in conjunction with the above-described processes to further define the binding site.

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

```
REM'RKS ATOMIC COORDINATES OF CD40L CRYSTAL STRUCTURE IN PDB FORMAT
CRYST    77.170   77.170   90.460  90.00  90.00 120.00 R3
ATOM      1   N   GLY   116      -7.954 -16.144  22.488  1.00 64.71      A
ATOM      2   HT1 GLY   116      -7.087 -15.852  21.964  1.00 15.00      A
ATOM      3   HT2 GLY   116      -8.082 -17.142  22.242  1.00 15.00      A
ATOM      4   HT3 GLY   116      -8.630 -15.576  21.928  1.00 15.00      A
ATOM      5   CA  GLY   116      -7.927 -15.755  23.928  1.00 64.37      A
ATOM      6   C   GLY   116      -6.990 -16.621  24.780  1.00 64.34      A
ATOM      7   O   GLY   116      -6.968 -17.814  24.563  1.00 64.44      A
ATOM      8   N   ASP   117      -6.238 -16.043  25.740  1.00 64.04      A
ATOM      9   H   ASP   117      -5.617 -16.709  26.170  1.00 15.00      A
ATOM     10   CA  ASP   117      -6.284 -14.616  26.130  1.00 63.57      A
ATOM     11   CB  ASP   117      -5.711 -14.402  27.539  1.00 63.36      A
ATOM     12   CG  ASP   117      -6.518 -15.163  28.574  1.00 63.71      A
ATOM     13   OD1 ASP   117      -6.090 -16.247  28.965  1.00 63.24      A
ATOM     14   OD2 ASP   117      -7.566 -14.668  28.987  1.00 63.29      A
ATOM     15   C   ASP   117      -5.651 -13.585  25.184  1.00 63.31      A
ATOM     16   O   ASP   117      -6.039 -12.427  25.145  1.00 63.35      A
ATOM     17   N   GLN   118      -4.713 -14.090  24.379  1.00 62.72      A
ATOM     18   H   GLN   118      -4.450 -15.040  24.541  1.00 15.00      A
ATOM     19   CA  GLN   118      -4.097 -13.313  23.281  1.00 61.79      A
ATOM     20   CB  GLN   118      -2.918 -14.117  22.687  1.00 62.46      A
ATOM     21   CG  GLN   118      -3.047 -15.659  22.562  1.00 62.95      A
ATOM     22   CD  GLN   118      -4.277 -16.118  21.790  1.00 63.26      A
ATOM     23   OE1 GLN   118      -5.396 -16.000  22.277  1.00 63.43      A
ATOM     24   NE2 GLN   118      -4.044 -16.665  20.601  1.00 63.42      A
ATOM     25   HE21 GLN  118      -4.836 -16.715  19.975  1.00 15.00      A
ATOM     26   HE22 GLN  118      -3.151 -16.995  20.298  1.00 15.00      A
ATOM     27   C   GLN   118      -4.999 -12.841  22.128  1.00 60.59      A
ATOM     28   O   GLN   118      -4.887 -13.379  21.052  1.00 60.79      A
ATOM     29   N   ASN   119      -5.912 -11.901  22.445  1.00 58.61      A
ATOM     30   H   ASN   119      -5.917 -11.600  23.389  1.00 15.00      A
ATOM     31   CA  ASN   119      -6.689 -11.222  21.386  1.00 56.39      A
ATOM     32   CB  ASN   119      -7.947 -11.982  20.936  1.00 56.95      A
ATOM     33   CG  ASN   119      -7.652 -13.352  20.375  1.00 57.45      A
ATOM     34   OD1 ASN   119      -7.941 -14.303  21.084  1.00 58.50      A
ATOM     35   ND2 ASN   119      -7.005 -13.431  19.241  1.00 58.58      A
ATOM     36   HD21 ASN  119      -6.843 -12.617  18.646  1.00 15.00      A
ATOM     37   HD22 ASN  119      -6.740 -14.221  18.684  1.00 15.00      A
ATOM     38   C   ASN   119      -7.053  -9.724  21.571  1.00 53.62      A
ATOM     39   O   ASN   119      -6.746  -8.933  20.694  1.00 56.55      A
ATOM     40   N   PRO   120      -7.737  -9.288  22.698  1.00 50.17      A
ATOM     41   CD  PRO   120      -8.151 -10.129  23.810  1.00 51.90      A
ATOM     42   CA  PRO   120      -8.402  -7.945  22.818  1.00 48.19      A
ATOM     43   CB  PRO   120      -9.191  -8.008  24.117  1.00 47.42      A
ATOM     44   CG  PRO   120      -9.444  -9.493  24.321  1.00 51.93      A
ATOM     45   C   PRO   120      -7.750  -6.524  22.657  1.00 45.59      A
ATOM     46   O   PRO   120      -8.187  -5.516  23.225  1.00 45.37      A
ATOM     47   N   GLN   121      -6.789  -6.458  21.721  1.00 38.52      A
ATOM     48   H   GLN   121      -6.287  -7.304  21.505  1.00 15.00      A
ATOM     49   CA  GLN   121      -6.733  -5.359  20.753  1.00 29.14      A
ATOM     50   CB  GLN   121      -5.454  -5.735  19.971  1.00 26.30      A
ATOM     51   CG  GLN   121      -5.128  -4.943  18.710  1.00 26.84      A
ATOM     52   CD  GLN   121      -4.923  -3.460  18.949  1.00 27.26      A
ATOM     53   OE1 GLN   121      -5.822  -2.668  18.709  1.00 28.66      A
ATOM     54   NE2 GLN   121      -3.717  -3.100  19.341  1.00 33.90      A
ATOM     55   HE21 GLN  121      -2.883  -3.614  19.564  1.00 15.00      A
ATOM     56   HE22 GLN  121      -3.442  -2.138  19.204  1.00 15.00      A
ATOM     57   C   GLN   121      -8.065  -5.218  19.903  1.00 26.33      A
ATOM     58   O   GLN   121      -8.905  -6.097  19.834  1.00 21.41      A
ATOM     59   N   ILE   122      -8.288  -4.051  19.272  1.00 21.21      A
```

Table 1

ATOM	60	H	ILE	122	-7.600	-3.320	19.337	1.00	15.00	A
ATOM	61	CA	ILE	122	-9.383	-3.952	18.295	1.00	20.92	A
ATOM	62	CB	ILE	122	-10.238	-2.629	18.396	1.00	22.17	A
ATOM	63	CG2	ILE	122	-11.275	-2.428	17.272	1.00	21.61	A
ATOM	64	CG1	ILE	122	-11.076	-2.744	19.668	1.00	24.13	A
ATOM	65	CD1	ILE	122	-11.751	-1.440	20.073	1.00	23.04	A
ATOM	66	C	ILE	122	-8.833	-4.108	16.895	1.00	18.96	A
ATOM	67	O	ILE	122	-8.135	-3.243	16.379	1.00	17.93	A
ATOM	68	N	ALA	123	-9.159	-5.240	16.283	1.00	14.72	A
ATOM	69	H	ALA	123	-9.599	-5.978	16.805	1.00	15.00	A
ATOM	70	CA	ALA	123	-8.656	-5.401	14.917	1.00	14.29	A
ATOM	71	CB	ALA	123	-7.176	-5.868	14.903	1.00	12.83	A
ATOM	72	C	ALA	123	-9.483	-6.315	13.985	1.00	15.66	A
ATOM	73	O	ALA	123	-10.170	-7.261	14.323	1.00	13.58	A
ATOM	74	N	ALA	124	-9.388	-6.009	12.724	1.00	13.45	A
ATOM	75	H	ALA	124	-8.894	-5.185	12.456	1.00	15.00	A
ATOM	76	CA	ALA	124	-10.087	-6.920	11.836	1.00	14.55	A
ATOM	77	CB	ALA	124	-11.486	-6.368	11.446	1.00	11.37	A
ATOM	78	C	ALA	124	-9.271	-7.123	10.563	1.00	13.54	A
ATOM	79	O	ALA	124	-8.501	-6.274	10.129	1.00	16.29	A
ATOM	80	N	HIS	125	-9.544	-8.248	9.937	1.00	11.49	A
ATOM	81	H	HIS	125	-10.100	-8.900	10.426	1.00	15.00	A
ATOM	82	CA	HIS	125	-9.100	-8.524	8.590	1.00	11.51	A
ATOM	83	CB	HIS	125	-7.605	-8.908	8.614	1.00	11.43	A
ATOM	84	CG	HIS	125	-7.119	-9.116	7.205	1.00	7.41	A
ATOM	85	ND1	HIS	125	-6.750	-8.130	6.421	1.00	6.60	A
ATOM	86	HD1	HIS	125	-6.708	-7.168	6.621	1.00	15.00	A
ATOM	87	CD2	HIS	125	-7.075	-10.291	6.456	1.00	12.36	A
ATOM	88	NE2	HIS	125	-6.670	-9.971	5.234	1.00	6.20	A
ATOM	89	CE1	HIS	125	-6.462	-8.646	5.211	1.00	4.48	A
ATOM	90	C	HIS	125	-10.024	-9.570	7.931	1.00	12.63	A
ATOM	91	O	HIS	125	-10.324	-10.650	8.383	1.00	13.14	A
ATOM	92	N	VAL	126	-10.550	-9.129	6.806	1.00	15.65	A
ATOM	93	H	VAL	126	-10.169	-8.286	6.428	1.00	15.00	A
ATOM	94	CA	VAL	126	-11.743	-9.717	6.201	1.00	14.38	A
ATOM	95	CB	VAL	126	-12.877	-8.808	6.675	1.00	13.37	A
ATOM	96	CG1	VAL	126	-13.794	-9.722	7.379	1.00	12.60	A
ATOM	97	CG2	VAL	126	-13.449	-7.663	5.814	1.00	9.61	A
ATOM	98	C	VAL	126	-11.502	-9.971	4.685	1.00	16.03	A
ATOM	99	O	VAL	126	-10.684	-9.297	4.074	1.00	16.42	A
ATOM	100	N	ILE	127	-12.118	-11.013	4.136	1.00	15.99	A
ATOM	101	H	ILE	127	-12.807	-11.481	4.691	1.00	15.00	A
ATOM	102	CA	ILE	127	-11.651	-11.532	2.831	1.00	14.86	A
ATOM	103	CB	ILE	127	-11.414	-13.051	3.002	1.00	17.56	A
ATOM	104	CG2	ILE	127	-11.716	-13.910	1.765	1.00	17.17	A
ATOM	105	CG1	ILE	127	-9.972	-13.316	3.399	1.00	16.47	A
ATOM	106	CD1	ILE	127	-9.705	-12.992	4.864	1.00	19.64	A
ATOM	107	C	ILE	127	-12.691	-11.269	1.765	1.00	18.96	A
ATOM	108	O	ILE	127	-13.898	-11.391	2.016	1.00	20.01	A
ATOM	109	N	SER	128	-12.229	-10.882	0.581	1.00	17.54	A
ATOM	110	H	SER	128	-11.232	-10.871	0.382	1.00	15.00	A
ATOM	111	CA	SER	128	-13.274	-10.667	-0.437	1.00	15.55	A
ATOM	112	CB	SER	128	-12.664	-10.130	-1.706	1.00	18.16	A
ATOM	113	OG	SER	128	-12.205	-11.207	-2.574	1.00	19.90	A
ATOM	114	HG	SER	128	-11.832	-11.931	-2.029	1.00	15.00	A
ATOM	115	C	SER	128	-14.295	-11.761	-0.792	1.00	13.62	A
ATOM	116	O	SER	128	-14.052	-12.960	-0.832	1.00	8.98	A
ATOM	117	N	GLU	129	-15.492	-11.246	-1.027	1.00	13.36	A
ATOM	118	H	GLU	129	-15.661	-10.257	-0.937	1.00	15.00	A
ATOM	119	CA	GLU	129	-16.379	-12.024	-1.840	1.00	17.20	A

ATOM	120	CB	GLU	129	-17.052	-13.117	-1.021	1.00 20.55	A
ATOM	121	CG	GLU	129	-18.092	-12.694	-0.036	1.00 17.92	A
ATOM	122	CD	GLU	129	-18.781	-13.951	0.376	1.00 21.98	A
ATOM	123	OE1	GLU	129	-19.997	-13.932	0.368	1.00 32.23	A
ATOM	124	OE2	GLU	129	-18.150	-14.938	0.734	1.00 33.12	A
ATOM	125	C	GLU	129	-17.371	-11.409	-2.809	1.00 17.71	A
ATOM	126	O	GLU	129	-17.972	-10.389	-2.553	1.00 21.59	A
ATOM	127	N	ALA	130	-17.550	-12.145	-3.914	1.00 20.52	A
ATOM	128	H	ALA	130	-17.136	-13.057	-3.923	1.00 15.00	A
ATOM	129	CA	ALA	130	-18.379	-11.649	-5.019	1.00 23.36	A
ATOM	130	CB	ALA	130	-18.424	-12.633	-6.208	1.00 19.66	A
ATOM	131	C	ALA	130	-19.811	-11.298	-4.570	1.00 26.86	A
ATOM	132	O	ALA	130	-20.519	-12.022	-3.869	1.00 29.40	A
ATOM	133	N	SER	131	-20.198	-10.086	-4.968	1.00 21.70	A
ATOM	134	H	SER	131	-19.515	-9.481	-5.410	1.00 15.00	A
ATOM	135	CA	SER	131	-21.592	-9.782	-4.732	1.00 20.04	A
ATOM	136	CB	SER	131	-21.829	-8.266	-4.787	1.00 20.65	A
ATOM	137	OG	SER	131	-23.182	-8.001	-4.435	1.00 15.24	A
ATOM	138	HG	SER	131	-23.329	-7.069	-4.559	1.00 15.00	A
ATOM	139	C	SER	131	-22.546	-10.501	-5.668	1.00 17.15	A
ATOM	140	O	SER	131	-22.236	-10.853	-6.786	1.00 14.30	A
ATOM	141	N	SER	132	-23.756	-10.731	-5.187	1.00 20.15	A
ATOM	142	H	SER	132	-23.967	-10.586	-4.209	1.00 15.00	A
ATOM	143	CA	SER	132	-24.674	-11.250	-6.218	1.00 21.62	A
ATOM	144	CB	SER	132	-25.266	-12.616	-5.893	1.00 16.00	A
ATOM	145	OG	SER	132	-26.203	-12.324	-4.894	1.00 23.84	A
ATOM	146	HG	SER	132	-26.016	-12.944	-4.179	1.00 15.00	A
ATOM	147	C	SER	132	-25.727	-10.268	-6.671	1.00 20.07	A
ATOM	148	O	SER	132	-26.535	-10.544	-7.547	1.00 20.27	A
ATOM	149	N	LYS	133	-25.606	-9.063	-6.118	1.00 21.87	A
ATOM	150	H	LYS	133	-24.904	-8.969	-5.397	1.00 15.00	A
ATOM	151	CA	LYS	133	-26.406	-7.916	-6.517	1.00 19.23	A
ATOM	152	CB	LYS	133	-27.024	-7.309	-5.256	1.00 23.08	A
ATOM	153	CG	LYS	133	-27.684	-8.364	-4.354	1.00 21.07	A
ATOM	154	CD	LYS	133	-29.174	-8.110	-4.330	1.00 27.36	A
ATOM	155	CE	LYS	133	-29.939	-7.884	-5.670	1.00 30.56	A
ATOM	156	NZ	LYS	133	-31.323	-7.515	-5.345	1.00 21.56	A
ATOM	157	HZ1	LYS	133	-31.862	-7.351	-6.218	1.00 15.00	A
ATOM	158	HZ2	LYS	133	-31.753	-8.299	-4.811	1.00 15.00	A
ATOM	159	HZ3	LYS	133	-31.333	-6.654	-4.760	1.00 15.00	A
ATOM	160	C	LYS	133	-25.579	-6.876	-7.194	1.00 20.10	A
ATOM	161	O	LYS	133	-24.378	-6.801	-7.007	1.00 17.94	A
ATOM	162	N	THR	134	-26.260	-6.052	-7.983	1.00 22.95	A
ATOM	163	H	THR	134	-27.275	-6.130	-8.036	1.00 15.00	A
ATOM	164	CA	THR	134	-25.556	-4.879	-8.561	1.00 27.89	A
ATOM	165	CB	THR	134	-26.498	-4.274	-9.592	1.00 24.59	A
ATOM	166	OG1	THR	134	-26.540	-5.037	-10.792	1.00 24.32	A
ATOM	167	HG1	THR	134	-26.232	-4.411	-11.456	1.00 15.00	A
ATOM	168	CG2	THR	134	-26.044	-2.897	-9.968	1.00 22.97	A
ATOM	169	C	THR	134	-24.987	-3.798	-7.559	1.00 32.51	A
ATOM	170	O	THR	134	-25.658	-3.461	-6.603	1.00 38.43	A
ATOM	171	N	THR	135	-23.717	-3.352	-7.690	1.00 35.98	A
ATOM	172	H	THR	135	-23.292	-3.555	-8.585	1.00 15.00	A
ATOM	173	CA	THR	135	-22.964	-3.469	-6.386	1.00 36.02	A
ATOM	174	CB	THR	135	-21.575	-4.276	-6.534	1.00 36.01	A
ATOM	175	OG1	THR	135	-21.645	-5.388	-7.488	1.00 30.60	A
ATOM	176	HG1	THR	135	-22.255	-6.094	-7.312	1.00 15.00	A
ATOM	177	CG2	THR	135	-20.866	-4.776	-5.264	1.00 35.55	A
ATOM	178	C	THR	135	-22.949	-2.266	-5.404	1.00 30.25	A
ATOM	179	O	THR	135	-23.541	-2.348	-4.331	1.00 28.35	A

ATOM	180	N	SER	136	-22.294	-1.146	-5.776	1.00	23.29	A
ATOM	181	H	SER	136	-22.828	-0.357	-5.460	1.00	15.00	A
ATOM	182	CA	SER	136	-20.857	-1.051	-6.143	1.00	23.04	A
ATOM	183	CB	SER	136	-20.560	0.187	-6.965	1.00	21.03	A
ATOM	184	OG	SER	136	-20.624	1.261	-6.043	1.00	28.21	A
ATOM	185	HG	SER	136	-19.815	1.793	-6.008	1.00	15.00	A
ATOM	186	C	SER	136	-19.853	-1.090	-4.958	1.00	21.77	A
ATOM	187	O	SER	136	-18.630	-1.096	-5.080	1.00	21.94	A
ATOM	188	N	VAL	137	-20.452	-1.227	-3.752	1.00	24.03	A
ATOM	189	H	VAL	137	-21.440	-1.063	-3.705	1.00	15.00	A
ATOM	190	CA	VAL	137	-19.699	-1.632	-2.570	1.00	19.65	A
ATOM	191	CB	VAL	137	-20.218	-1.010	-1.248	1.00	21.14	A
ATOM	192	CG1	VAL	137	-20.419	-1.907	-0.058	1.00	18.16	A
ATOM	193	CG2	VAL	137	-21.322	-0.026	-1.442	1.00	13.49	A
ATOM	194	C	VAL	137	-19.370	-3.116	-2.473	1.00	17.15	A
ATOM	195	O	VAL	137	-20.209	-3.969	-2.593	1.00	16.69	A
ATOM	196	N	LEU	138	-18.077	-3.344	-2.271	1.00	15.84	A
ATOM	197	H	LEU	138	-17.502	-2.528	-2.246	1.00	15.00	A
ATOM	198	CA	LEU	138	-17.507	-4.667	-1.938	1.00	18.21	A
ATOM	199	CB	LEU	138	-15.962	-4.530	-1.791	1.00	13.60	A
ATOM	200	CG	LEU	138	-15.273	-3.854	-2.998	1.00	16.09	A
ATOM	201	CD1	LEU	138	-15.923	-4.379	-4.300	1.00	20.35	A
ATOM	202	CD2	LEU	138	-13.710	-3.936	-2.982	1.00	12.34	A
ATOM	203	C	LEU	138	-18.170	-5.480	-0.772	1.00	16.29	A
ATOM	204	O	LEU	138	-18.498	-4.986	0.301	1.00	12.97	A
ATOM	205	N	GLN	139	-18.345	-6.768	-1.035	1.00	13.04	A
ATOM	206	H	GLN	139	-18.052	-7.078	-1.960	1.00	15.00	A
ATOM	207	CA	GLN	139	-18.757	-7.658	0.013	1.00	15.32	A
ATOM	208	CB	GLN	139	-19.847	-8.678	-0.481	1.00	13.99	A
ATOM	209	CG	GLN	139	-21.068	-7.960	-1.113	1.00	20.85	A
ATOM	210	CD	GLN	139	-21.872	-7.022	-0.193	1.00	22.04	A
ATOM	211	OE1	GLN	139	-22.343	-7.439	0.878	1.00	25.45	A
ATOM	212	NE2	GLN	139	-21.963	-5.739	-0.618	1.00	17.74	A
ATOM	213	HE21	GLN	139	-22.697	-5.181	-0.206	1.00	15.00	A
ATOM	214	HE22	GLN	139	-21.460	-5.326	-1.374	1.00	15.00	A
ATOM	215	C	GLN	139	-17.527	-8.383	0.541	1.00	14.26	A
ATOM	216	O	GLN	139	-16.554	-8.640	-0.144	1.00	14.40	A
ATOM	217	N	TRP	140	-17.647	-8.780	1.805	1.00	12.80	A
ATOM	218	H	TRP	140	-18.433	-8.447	2.297	1.00	15.00	A
ATOM	219	CA	TRP	140	-16.542	-9.500	2.463	1.00	14.03	A
ATOM	220	CB	TRP	140	-15.813	-8.623	3.483	1.00	14.18	A
ATOM	221	CG	TRP	140	-15.467	-7.291	2.823	1.00	8.44	A
ATOM	222	CD2	TRP	140	-14.379	-6.966	1.941	1.00	9.01	A
ATOM	223	CE2	TRP	140	-14.549	-5.625	1.482	1.00	8.40	A
ATOM	224	CE3	TRP	140	-13.215	-7.688	1.581	1.00	10.14	A
ATOM	225	CD1	TRP	140	-16.225	-6.137	2.863	1.00	11.29	A
ATOM	226	NE1	TRP	140	-15.710	-5.150	2.077	1.00	14.27	A
ATOM	227	HE1	TRP	140	-16.121	-4.268	2.010	1.00	15.00	A
ATOM	228	CZ2	TRP	140	-13.640	-5.009	0.590	1.00	8.16	A
ATOM	229	CZ3	TRP	140	-12.292	-7.069	0.713	1.00	13.90	A
ATOM	230	CH2	TRP	140	-12.497	-5.749	0.215	1.00	12.11	A
ATOM	231	C	TRP	140	-17.016	-10.701	3.170	1.00	14.34	A
ATOM	232	O	TRP	140	-18.193	-10.862	3.392	1.00	16.00	A
ATOM	233	N	ALA	141	-16.082	-11.528	3.558	1.00	14.80	A
ATOM	234	H	ALA	141	-15.133	-11.377	3.294	1.00	15.00	A
ATOM	235	CA	ALA	141	-16.489	-12.617	4.394	1.00	15.27	A
ATOM	236	CB	ALA	141	-16.504	-13.920	3.583	1.00	16.97	A
ATOM	237	C	ALA	141	-15.585	-12.761	5.607	1.00	15.90	A
ATOM	238	O	ALA	141	-14.453	-12.338	5.550	1.00	14.25	A
ATOM	239	N	GLU	142	-16.068	-13.366	6.688	1.00	19.74	A

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ATOM    240  H   GLU   142     -17.055 -13.574   6.688  1.00 15.00      A
ATOM    241  CA  GLU   142     -15.149 -13.759   7.731  1.00 25.93      A
ATOM    242  CB  GLU   142     -15.794 -13.910   9.117  1.00 21.75      A
ATOM    243  CG  GLU   142     -15.716 -12.456   9.647  1.00 24.05      A
ATOM    244  CD  GLU   142     -16.749 -12.087  10.711  1.00 26.61      A
ATOM    245  OE1 GLU   142     -17.908 -11.888  10.361  1.00 34.72      A
ATOM    246  OE2 GLU   142     -16.404 -11.984  11.886  1.00 30.07      A
ATOM    247  C   GLU   142     -14.200 -14.797   7.193  1.00 33.25      A
ATOM    248  O   GLU   142     -13.156 -14.349   6.737  1.00 41.84      A
ATOM    249  N   LYS   143     -14.577 -16.080   7.084  1.00 34.17      A
ATOM    250  H   LYS   143     -15.432 -16.384   7.492  1.00 15.00      A
ATOM    251  CA  LYS   143     -13.882 -16.854   5.980  1.00 35.31      A
ATOM    252  CB  LYS   143     -14.673 -16.603   4.681  1.00 37.64      A
ATOM    253  CG  LYS   143     -14.300 -17.505   3.531  1.00 47.37      A
ATOM    254  CD  LYS   143     -15.022 -17.284   2.202  1.00 50.37      A
ATOM    255  CE  LYS   143     -14.686 -16.047   1.357  1.00 49.23      A
ATOM    256  NZ  LYS   143     -15.632 -16.097   0.221  1.00 51.67      A
ATOM    257  HZ1 LYS   143     -15.333 -15.445  -0.534  1.00 15.00      A
ATOM    258  HZ2 LYS   143     -15.680 -17.061  -0.177  1.00 15.00      A
ATOM    259  HZ3 LYS   143     -16.564 -15.833   0.585  1.00 15.00      A
ATOM    260  C   LYS   143     -12.330 -16.979   5.637  1.00 32.80      A
ATOM    261  O   LYS   143     -11.831 -18.041   5.276  1.00 35.64      A
ATOM    262  N   GLY   144     -11.522 -15.923   5.637  1.00 28.26      A
ATOM    263  H   GLY   144     -11.718 -14.995   5.910  1.00 15.00      A
ATOM    264  CA  GLY   144     -10.243 -16.458   5.194  1.00 32.94      A
ATOM    265  C   GLY   144      -9.178 -16.862   6.180  1.00 29.93      A
ATOM    266  O   GLY   144      -9.345 -17.454   7.205  1.00 24.67      A
ATOM    267  N   TYR   145      -8.069 -16.270   5.815  1.00 26.37      A
ATOM    268  H   TYR   145      -8.160 -15.729   4.966  1.00 15.00      A
ATOM    269  CA  TYR   145      -7.027 -16.002   6.777  1.00 27.61      A
ATOM    270  CB  TYR   145      -5.708 -15.877   5.947  1.00 37.54      A
ATOM    271  CG  TYR   145      -5.962 -15.774   4.456  1.00 50.95      A
ATOM    272  CD1 TYR   145      -5.682 -14.633   3.706  1.00 53.22      A
ATOM    273  CE1 TYR   145      -6.313 -14.377   2.468  1.00 60.28      A
ATOM    274  CD2 TYR   145      -6.591 -16.847   3.791  1.00 53.11      A
ATOM    275  CE2 TYR   145      -7.207 -16.699   2.551  1.00 56.30      A
ATOM    276  CZ  TYR   145      -7.162 -15.430   1.873  1.00 61.12      A
ATOM    277  OH  TYR   145      -7.812 -15.119   0.665  1.00 62.63      A
ATOM    278  HH  TYR   145      -8.575 -15.686   0.401  1.00 15.00      A
ATOM    279  C   TYR   145      -7.532 -14.762   7.620  1.00 22.41      A
ATOM    280  O   TYR   145      -7.000 -13.677   7.650  1.00 22.68      A
ATOM    281  N   TYR   146      -8.731 -14.884   8.196  1.00 20.39      A
ATOM    282  H   TYR   146      -8.935 -15.824   8.509  1.00 15.00      A
ATOM    283  CA  TYR   146      -9.423 -13.700   8.725  1.00 20.40      A
ATOM    284  CB  TYR   146     -10.886 -13.673   8.306  1.00 22.53      A
ATOM    285  CG  TYR   146     -11.710 -14.460   9.286  1.00 23.02      A
ATOM    286  CD1 TYR   146     -11.635 -15.873   9.236  1.00 26.99      A
ATOM    287  CE1 TYR   146     -12.254 -16.623  10.239  1.00 25.44      A
ATOM    288  CD2 TYR   146     -12.477 -13.766  10.236  1.00 23.45      A
ATOM    289  CE2 TYR   146     -13.150 -14.520  11.205  1.00 26.81      A
ATOM    290  CZ  TYR   146     -13.007 -15.937  11.204  1.00 27.40      A
ATOM    291  OH  TYR   146     -13.647 -16.689  12.170  1.00 31.91      A
ATOM    292  HH  TYR   146     -12.911 -17.080  12.676  1.00 15.00      A
ATOM    293  C   TYR   146      -9.291 -13.419  10.219  1.00 18.79      A
ATOM    294  O   TYR   146      -8.904 -14.232  11.012  1.00 16.13      A
ATOM    295  N   THR   147      -9.596 -12.169  10.556  1.00 17.54      A
ATOM    296  H   THR   147      -9.973 -11.607   9.830  1.00 15.00      A
ATOM    297  CA  THR   147      -9.432 -11.764  11.948  1.00 14.06      A
ATOM    298  CB  THR   147      -8.162 -10.875  12.182  1.00 13.66      A
ATOM    299  OG1 THR   147      -6.912 -11.505  11.856  1.00 12.56      A
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ATOM	300	HG1	THR	147	-6.934	-11.898	10.980	1.00	15.00	A
ATOM	301	CG2	THR	147	-8.025	-10.236	13.554	1.00	7.22	A
ATOM	302	C	THR	147	-10.619	-10.925	12.253	1.00	15.60	A
ATOM	303	O	THR	147	-11.044	-10.074	11.496	1.00	16.39	A
ATOM	304	N	MET	148	-11.144	-11.139	13.412	1.00	20.67	A
ATOM	305	H	MET	148	-10.838	-11.988	13.828	1.00	15.00	A
ATOM	306	CA	MET	148	-12.124	-10.311	14.110	1.00	19.71	A
ATOM	307	CB	MET	148	-13.546	-10.702	13.705	1.00	17.89	A
ATOM	308	CG	MET	148	-14.541	-9.580	14.019	1.00	13.53	A
ATOM	309	SD	MET	148	-14.492	-8.149	12.952	1.00	14.69	A
ATOM	310	CE	MET	148	-14.566	-8.928	11.333	1.00	10.10	A
ATOM	311	C	MET	148	-11.915	-10.282	15.639	1.00	21.49	A
ATOM	312	O	MET	148	-12.594	-10.905	16.436	1.00	22.98	A
ATOM	313	N	SER	149	-10.955	-9.412	16.055	1.00	20.58	A
ATOM	314	H	SER	149	-10.516	-8.786	15.406	1.00	15.00	A
ATOM	315	CA	SER	149	-10.388	-9.698	17.419	1.00	19.11	A
ATOM	316	CB	SER	149	-9.174	-8.860	17.792	1.00	12.17	A
ATOM	317	OG	SER	149	-9.540	-7.513	17.975	1.00	14.10	A
ATOM	318	HG	SER	149	-9.571	-7.487	18.934	1.00	15.00	A
ATOM	319	C	SER	149	-11.203	-9.844	18.727	1.00	22.19	A
ATOM	320	O	SER	149	-10.728	-10.267	19.772	1.00	22.95	A
ATOM	321	N	ASN	150	-12.456	-9.322	18.631	1.00	22.71	A
ATOM	322	H	ASN	150	-12.782	-9.247	17.688	1.00	15.00	A
ATOM	323	CA	ASN	150	-13.361	-9.236	19.764	1.00	20.32	A
ATOM	324	CB	ASN	150	-12.734	-8.446	20.955	1.00	21.56	A
ATOM	325	CG	ASN	150	-12.343	-6.962	20.706	1.00	20.71	A
ATOM	326	OD1	ASN	150	-13.059	-6.187	20.119	1.00	17.81	A
ATOM	327	ND2	ASN	150	-11.222	-6.485	21.271	1.00	23.86	A
ATOM	328	HD21	ASN	150	-11.035	-5.521	21.092	1.00	15.00	A
ATOM	329	HD22	ASN	150	-10.670	-7.109	21.821	1.00	15.00	A
ATOM	330	C	ASN	150	-14.644	-8.657	19.256	1.00	20.60	A
ATOM	331	O	ASN	150	-14.718	-8.130	18.148	1.00	20.56	A
ATOM	332	N	ASN	151	-15.637	-8.713	20.149	1.00	23.49	A
ATOM	333	H	ASN	151	-15.455	-9.124	21.038	1.00	15.00	A
ATOM	334	CA	ASN	151	-16.974	-8.080	19.823	1.00	24.71	A
ATOM	335	CB	ASN	151	-18.130	-8.645	20.712	1.00	28.30	A
ATOM	336	CG	ASN	151	-17.959	-8.271	22.173	1.00	33.23	A
ATOM	337	OD1	ASN	151	-17.075	-7.562	22.606	1.00	39.79	A
ATOM	338	ND2	ASN	151	-18.782	-8.838	23.011	1.00	38.32	A
ATOM	339	HD21	ASN	151	-18.553	-8.524	23.928	1.00	15.00	A
ATOM	340	HD22	ASN	151	-19.495	-9.465	22.733	1.00	15.00	A
ATOM	341	C	ASN	151	-17.172	-6.531	19.645	1.00	22.53	A
ATOM	342	O	ASN	151	-18.254	-6.048	19.374	1.00	21.32	A
ATOM	343	N	LEU	152	-16.066	-5.762	19.859	1.00	23.00	A
ATOM	344	H	LEU	152	-15.247	-6.289	20.070	1.00	15.00	A
ATOM	345	CA	LEU	152	-15.924	-4.335	19.525	1.00	18.87	A
ATOM	346	CB	LEU	152	-14.830	-3.700	20.325	1.00	21.77	A
ATOM	347	CG	LEU	152	-14.981	-3.999	21.806	1.00	24.80	A
ATOM	348	CD1	LEU	152	-16.390	-3.645	22.316	1.00	22.82	A
ATOM	349	CD2	LEU	152	-13.847	-3.256	22.556	1.00	23.56	A
ATOM	350	C	LEU	152	-15.565	-3.993	18.094	1.00	17.34	A
ATOM	351	O	LEU	152	-15.590	-2.840	17.708	1.00	13.39	A
ATOM	352	N	VAL	153	-15.267	-5.054	17.309	1.00	18.65	A
ATOM	353	H	VAL	153	-15.156	-5.962	17.716	1.00	15.00	A
ATOM	354	CA	VAL	153	-15.439	-4.910	15.849	1.00	16.81	A
ATOM	355	CB	VAL	153	-14.138	-5.021	14.980	1.00	15.33	A
ATOM	356	CG1	VAL	153	-12.908	-5.718	15.562	1.00	21.22	A
ATOM	357	CG2	VAL	153	-13.775	-3.757	14.287	1.00	16.95	A
ATOM	358	C	VAL	153	-16.405	-5.964	15.301	1.00	13.48	A
ATOM	359	O	VAL	153	-16.363	-7.116	15.647	1.00	13.06	A

ATOM	360	N	THR	154	-17.207	-5.546	14.358	1.00	12.06	A
ATOM	361	H	THR	154	-17.313	-4.568	14.215	1.00	15.00	A
ATOM	362	CA	THR	154	-17.903	-6.600	13.615	1.00	16.26	A
ATOM	363	CB	THR	154	-19.366	-6.747	14.157	1.00	19.51	A
ATOM	364	OG1	THR	154	-19.995	-5.459	14.205	1.00	19.31	A
ATOM	365	HG1	THR	154	-20.577	-5.508	14.949	1.00	15.00	A
ATOM	366	CG2	THR	154	-19.502	-7.288	15.571	1.00	21.62	A
ATOM	367	C	THR	154	-17.997	-6.252	12.107	1.00	18.12	A
ATOM	368	O	THR	154	-17.992	-5.110	11.605	1.00	16.55	A
ATOM	369	N	LEU	155	-18.101	-7.324	11.357	1.00	16.77	A
ATOM	370	H	LEU	155	-18.056	-8.202	11.791	1.00	15.00	A
ATOM	371	CA	LEU	155	-18.514	-7.198	9.967	1.00	17.10	A
ATOM	372	CB	LEU	155	-17.829	-8.353	9.204	1.00	20.04	A
ATOM	373	CG	LEU	155	-17.524	-8.428	7.692	1.00	20.81	A
ATOM	374	CD1	LEU	155	-17.822	-7.159	6.908	1.00	17.03	A
ATOM	375	CD2	LEU	155	-17.912	-9.810	7.139	1.00	12.42	A
ATOM	376	C	LEU	155	-20.055	-7.187	9.904	1.00	20.71	A
ATOM	377	O	LEU	155	-20.712	-8.163	10.217	1.00	18.01	A
ATOM	378	N	GLU	156	-20.593	-5.995	9.561	1.00	19.51	A
ATOM	379	H	GLU	156	-19.959	-5.230	9.440	1.00	15.00	A
ATOM	380	CA	GLU	156	-22.036	-5.888	9.413	1.00	21.95	A
ATOM	381	CB	GLU	156	-22.641	-4.631	10.033	1.00	18.95	A
ATOM	382	CG	GLU	156	-22.098	-4.412	11.436	1.00	27.68	A
ATOM	383	CD	GLU	156	-22.721	-5.194	12.587	1.00	31.62	A
ATOM	384	OE1	GLU	156	-23.347	-6.248	12.367	1.00	33.40	A
ATOM	385	OE2	GLU	156	-22.532	-4.721	13.724	1.00	35.00	A
ATOM	386	C	GLU	156	-22.457	-5.966	7.964	1.00	25.36	A
ATOM	387	O	GLU	156	-21.958	-5.298	7.077	1.00	22.70	A
ATOM	388	N	ASN	157	-23.437	-6.808	7.696	1.00	30.92	A
ATOM	389	H	ASN	157	-23.594	-7.590	8.300	1.00	15.00	A
ATOM	390	CA	ASN	157	-23.804	-6.620	6.300	1.00	33.31	A
ATOM	391	CB	ASN	157	-23.856	-7.970	5.614	1.00	31.69	A
ATOM	392	CG	ASN	157	-23.669	-7.693	4.168	1.00	27.70	A
ATOM	393	OD1	ASN	157	-23.397	-6.593	3.810	1.00	25.89	A
ATOM	394	ND2	ASN	157	-23.893	-8.640	3.275	1.00	41.69	A
ATOM	395	HD21	ASN	157	-24.069	-9.603	3.467	1.00	15.00	A
ATOM	396	HD22	ASN	157	-23.745	-8.295	2.340	1.00	15.00	A
ATOM	397	C	ASN	157	-24.988	-5.658	6.118	1.00	35.08	A
ATOM	398	O	ASN	157	-26.107	-5.949	6.499	1.00	37.06	A
ATOM	399	N	GLY	158	-24.746	-4.443	5.560	1.00	40.03	A
ATOM	400	H	GLY	158	-25.601	-3.952	5.429	1.00	15.00	A
ATOM	401	CA	GLY	158	-23.422	-3.887	5.121	1.00	38.11	A
ATOM	402	C	GLY	158	-23.062	-3.720	3.617	1.00	37.48	A
ATOM	403	O	GLY	158	-23.890	-3.108	2.950	1.00	41.11	A
ATOM	404	N	LYS	159	-21.867	-4.220	3.135	1.00	32.75	A
ATOM	405	H	LYS	159	-21.904	-4.134	2.130	1.00	15.00	A
ATOM	406	CA	LYS	159	-20.828	-4.928	3.962	1.00	27.83	A
ATOM	407	CB	LYS	159	-20.317	-6.122	3.217	1.00	28.17	A
ATOM	408	CG	LYS	159	-19.734	-7.168	4.069	1.00	20.48	A
ATOM	409	CD	LYS	159	-20.533	-8.426	4.192	1.00	29.61	A
ATOM	410	CE	LYS	159	-20.577	-9.191	2.869	1.00	40.41	A
ATOM	411	NZ	LYS	159	-20.796	-10.663	2.986	1.00	40.88	A
ATOM	412	HZ1	LYS	159	-20.739	-11.087	2.035	1.00	15.00	A
ATOM	413	HZ2	LYS	159	-20.070	-11.087	3.600	1.00	15.00	A
ATOM	414	HZ3	LYS	159	-21.738	-10.848	3.389	1.00	15.00	A
ATOM	415	C	LYS	159	-19.688	-4.065	4.463	1.00	26.08	A
ATOM	416	O	LYS	159	-19.023	-3.369	3.696	1.00	28.01	A
ATOM	417	N	GLN	160	-19.683	-3.990	5.807	1.00	18.90	A
ATOM	418	H	GLN	160	-20.211	-4.674	6.319	1.00	15.00	A
ATOM	419	CA	GLN	160	-18.922	-2.929	6.464	1.00	13.89	A

ATOM	420	CB	GLN	160	-19.778	-1.694	6.611	1.00	16.79	A
ATOM	421	CG	GLN	160	-20.881	-1.896	7.633	1.00	18.34	A
ATOM	422	CD	GLN	160	-22.133	-1.166	7.193	1.00	23.97	A
ATOM	423	OE1	GLN	160	-23.088	-0.970	7.893	1.00	31.18	A
ATOM	424	NE2	GLN	160	-22.257	-0.771	5.948	1.00	28.16	A
ATOM	425	HE21	GLN	160	-23.194	-0.420	5.928	1.00	15.00	A
ATOM	426	HE22	GLN	160	-21.624	-0.780	5.186	1.00	15.00	A
ATOM	427	C	GLN	160	-18.313	-3.309	7.777	1.00	12.87	A
ATOM	428	O	GLN	160	-18.838	-4.151	8.498	1.00	14.78	A
ATOM	429	N	LEU	161	-17.187	-2.637	8.085	1.00	11.22	A
ATOM	430	H	LEU	161	-16.767	-2.124	7.340	1.00	15.00	A
ATOM	431	CA	LEU	161	-16.583	-2.870	9.405	1.00	9.71	A
ATOM	432	CB	LEU	161	-15.052	-2.939	9.390	1.00	4.67	A
ATOM	433	CG	LEU	161	-14.438	-4.060	8.559	1.00	7.30	A
ATOM	434	CD1	LEU	161	-14.511	-5.447	9.207	1.00	10.80	A
ATOM	435	CD2	LEU	161	-12.964	-3.794	8.389	1.00	5.48	A
ATOM	436	C	LEU	161	-17.082	-1.836	10.412	1.00	10.17	A
ATOM	437	O	LEU	161	-16.826	-0.657	10.341	1.00	13.36	A
ATOM	438	N	THR	162	-17.848	-2.338	11.375	1.00	16.94	A
ATOM	439	H	THR	162	-18.153	-3.279	11.251	1.00	15.00	A
ATOM	440	CA	THR	162	-18.317	-1.480	12.493	1.00	16.14	A
ATOM	441	CB	THR	162	-19.807	-1.769	12.640	1.00	13.33	A
ATOM	442	OG1	THR	162	-20.339	-1.707	11.308	1.00	16.73	A
ATOM	443	HG1	THR	162	-21.211	-1.254	11.343	1.00	15.00	A
ATOM	444	CG2	THR	162	-20.553	-0.832	13.562	1.00	15.01	A
ATOM	445	C	THR	162	-17.531	-1.547	13.842	1.00	13.28	A
ATOM	446	O	THR	162	-17.358	-2.587	14.449	1.00	20.21	A
ATOM	447	N	VAL	163	-16.994	-0.437	14.282	1.00	14.22	A
ATOM	448	H	VAL	163	-16.859	0.243	13.567	1.00	15.00	A
ATOM	449	CA	VAL	163	-16.326	-0.358	15.586	1.00	15.72	A
ATOM	450	CB	VAL	163	-15.038	0.426	15.428	1.00	11.82	A
ATOM	451	CG1	VAL	163	-15.191	1.944	15.368	1.00	9.87	A
ATOM	452	CG2	VAL	163	-14.229	-0.124	14.245	1.00	18.88	A
ATOM	453	C	VAL	163	-17.193	0.283	16.706	1.00	17.93	A
ATOM	454	O	VAL	163	-18.001	1.180	16.453	1.00	20.25	A
ATOM	455	N	LYS	164	-17.037	-0.232	17.925	1.00	15.44	A
ATOM	456	H	LYS	164	-16.254	-0.858	18.020	1.00	15.00	A
ATOM	457	CA	LYS	164	-17.856	0.138	19.109	1.00	17.33	A
ATOM	458	CB	LYS	164	-18.351	-1.150	19.807	1.00	19.58	A
ATOM	459	CG	LYS	164	-19.214	-1.885	18.759	1.00	23.56	A
ATOM	460	CD	LYS	164	-19.417	-3.410	18.851	1.00	28.85	A
ATOM	461	CE	LYS	164	-20.039	-4.047	17.554	1.00	33.81	A
ATOM	462	NZ	LYS	164	-19.428	-3.681	16.227	1.00	18.98	A
ATOM	463	HZ1	LYS	164	-19.195	-2.667	16.222	1.00	15.00	A
ATOM	464	HZ2	LYS	164	-18.552	-4.223	16.092	1.00	15.00	A
ATOM	465	HZ3	LYS	164	-20.084	-3.888	15.445	1.00	15.00	A
ATOM	466	C	LYS	164	-17.193	1.099	20.056	1.00	15.14	A
ATOM	467	O	LYS	164	-17.712	1.588	21.048	1.00	17.72	A
ATOM	468	N	ARG	165	-15.992	1.428	19.621	1.00	17.49	A
ATOM	469	H	ARG	165	-15.550	0.838	18.932	1.00	15.00	A
ATOM	470	CA	ARG	165	-15.184	2.415	20.325	1.00	20.18	A
ATOM	471	CB	ARG	165	-13.985	1.806	21.049	1.00	24.65	A
ATOM	472	CG	ARG	165	-14.363	0.833	22.126	1.00	29.54	A
ATOM	473	CD	ARG	165	-13.274	1.077	23.145	1.00	38.82	A
ATOM	474	NE	ARG	165	-13.719	1.998	24.186	1.00	43.41	A
ATOM	475	HE	ARG	165	-14.331	1.671	24.908	1.00	15.00	A
ATOM	476	CZ	ARG	165	-13.190	3.250	24.362	1.00	44.06	A
ATOM	477	NH1	ARG	165	-13.406	3.765	25.562	1.00	41.25	A
ATOM	478	HH11	ARG	165	-13.054	4.683	25.763	1.00	15.00	A
ATOM	479	HH12	ARG	165	-13.919	3.249	26.250	1.00	15.00	A

ATOM	480	NH2	ARG	165	-12.485	3.946	23.425	1.00	31.65	A
ATOM	481	HH21	ARG	165	-12.133	4.860	23.623	1.00	15.00	A
ATOM	482	HH22	ARG	165	-12.322	3.527	22.530	1.00	15.00	A
ATOM	483	C	ARG	165	-14.608	3.554	19.510	1.00	17.70	A
ATOM	484	O	ARG	165	-14.018	3.450	18.441	1.00	18.26	A
ATOM	485	N	GLN	166	-14.763	4.687	20.151	1.00	17.43	A
ATOM	486	H	GLN	166	-15.263	4.614	21.007	1.00	15.00	A
ATOM	487	CA	GLN	166	-14.138	5.911	19.698	1.00	19.00	A
ATOM	488	CB	GLN	166	-14.613	7.021	20.610	1.00	23.79	A
ATOM	489	CG	GLN	166	-14.067	8.409	20.386	1.00	34.06	A
ATOM	490	CD	GLN	166	-15.178	9.399	20.659	1.00	45.91	A
ATOM	491	OE1	GLN	166	-15.102	10.492	20.135	1.00	53.64	A
ATOM	492	NE2	GLN	166	-16.202	9.046	21.418	1.00	44.10	A
ATOM	493	HE21	GLN	166	-16.906	9.765	21.443	1.00	15.00	A
ATOM	494	HE22	GLN	166	-16.577	8.287	21.935	1.00	15.00	A
ATOM	495	C	GLN	166	-12.649	5.881	19.644	1.00	17.48	A
ATOM	496	O	GLN	166	-12.029	5.378	20.561	1.00	18.13	A
ATOM	497	N	GLY	167	-12.160	6.478	18.565	1.00	14.83	A
ATOM	498	H	GLY	167	-12.750	6.836	17.850	1.00	15.00	A
ATOM	499	CA	GLY	167	-10.728	6.711	18.557	1.00	16.28	A
ATOM	500	C	GLY	167	-10.044	6.685	17.204	1.00	16.48	A
ATOM	501	O	GLY	167	-10.674	6.601	16.162	1.00	19.19	A
ATOM	502	N	LEU	168	-8.720	6.735	17.209	1.00	17.06	A
ATOM	503	H	LEU	168	-8.311	6.890	18.120	1.00	15.00	A
ATOM	504	CA	LEU	168	-7.925	6.625	15.992	1.00	16.60	A
ATOM	505	CB	LEU	168	-6.600	7.343	16.289	1.00	21.87	A
ATOM	506	CG	LEU	168	-6.247	8.745	15.716	1.00	22.69	A
ATOM	507	CD1	LEU	168	-5.119	9.410	16.539	1.00	21.20	A
ATOM	508	CD2	LEU	168	-7.436	9.617	15.361	1.00	18.38	A
ATOM	509	C	LEU	168	-7.686	5.136	15.604	1.00	14.84	A
ATOM	510	O	LEU	168	-7.282	4.278	16.392	1.00	15.89	A
ATOM	511	N	TYR	169	-7.943	4.873	14.300	1.00	10.57	A
ATOM	512	H	TYR	169	-8.313	5.659	13.807	1.00	15.00	A
ATOM	513	CA	TYR	169	-7.683	3.572	13.656	1.00	5.27	A
ATOM	514	CB	TYR	169	-8.989	3.014	13.230	1.00	5.83	A
ATOM	515	CG	TYR	169	-9.857	2.620	14.423	1.00	6.94	A
ATOM	516	CD1	TYR	169	-10.524	3.598	15.168	1.00	7.40	A
ATOM	517	CE1	TYR	169	-11.390	3.193	16.218	1.00	7.77	A
ATOM	518	CD2	TYR	169	-10.016	1.255	14.744	1.00	8.89	A
ATOM	519	CE2	TYR	169	-10.850	0.841	15.804	1.00	9.40	A
ATOM	520	CZ	TYR	169	-11.563	1.827	16.534	1.00	10.39	A
ATOM	521	OH	TYR	169	-12.443	1.410	17.534	1.00	7.99	A
ATOM	522	HH	TYR	169	-13.009	2.117	17.800	1.00	15.00	A
ATOM	523	C	TYR	169	-6.810	3.642	12.390	1.00	6.72	A
ATOM	524	O	TYR	169	-6.917	4.498	11.557	1.00	9.12	A
ATOM	525	N	TYR	170	-5.899	2.722	12.228	1.00	9.53	A
ATOM	526	H	TYR	170	-5.806	2.081	12.986	1.00	15.00	A
ATOM	527	CA	TYR	170	-5.313	2.511	10.899	1.00	10.01	A
ATOM	528	CB	TYR	170	-3.967	1.797	11.044	1.00	7.46	A
ATOM	529	CG	TYR	170	-3.259	1.636	9.679	1.00	13.45	A
ATOM	530	CD1	TYR	170	-2.680	2.766	9.052	1.00	12.66	A
ATOM	531	CE1	TYR	170	-2.213	2.658	7.738	1.00	10.18	A
ATOM	532	CD2	TYR	170	-3.304	0.385	9.057	1.00	10.90	A
ATOM	533	CE2	TYR	170	-2.891	0.303	7.730	1.00	8.68	A
ATOM	534	CZ	TYR	170	-2.331	1.419	7.124	1.00	9.97	A
ATOM	535	OH	TYR	170	-1.774	1.286	5.859	1.00	17.50	A
ATOM	536	HH	TYR	170	-1.886	0.404	5.514	1.00	15.00	A
ATOM	537	C	TYR	170	-6.279	1.610	10.073	1.00	10.40	A
ATOM	538	O	TYR	170	-6.679	0.500	10.421	1.00	12.52	A
ATOM	539	N	ILE	171	-6.704	2.174	8.968	1.00	12.16	A

ATOM	540	H	ILE	171	-6.475	3.135	8.808	1.00 15.00	A
ATOM	541	CA	ILE	171	-7.608	1.430	8.138	1.00 9.37	A
ATOM	542	CB	ILE	171	-9.070	1.990	8.317	1.00 11.21	A
ATOM	543	CG2	ILE	171	-9.326	3.501	8.677	1.00 17.27	A
ATOM	544	CG1	ILE	171	-10.046	1.564	7.214	1.00 13.33	A
ATOM	545	CD1	ILE	171	-10.647	0.250	7.619	1.00 17.53	A
ATOM	546	C	ILE	171	-7.074	1.234	6.694	1.00 8.34	A
ATOM	547	O	ILE	171	-6.453	2.088	6.082	1.00 6.96	A
ATOM	548	N	TYR	172	-7.286	0.005	6.216	1.00 11.07	A
ATOM	549	H	TYR	172	-7.809	-0.624	6.786	1.00 15.00	A
ATOM	550	CA	TYR	172	-6.708	-0.378	4.922	1.00 15.60	A
ATOM	551	CB	TYR	172	-5.332	-1.082	5.037	1.00 14.32	A
ATOM	552	CG	TYR	172	-5.389	-2.397	5.796	1.00 9.21	A
ATOM	553	CD1	TYR	172	-5.342	-2.402	7.216	1.00 12.52	A
ATOM	554	CE1	TYR	172	-5.607	-3.620	7.901	1.00 10.88	A
ATOM	555	CD2	TYR	172	-5.565	-3.586	5.050	1.00 12.66	A
ATOM	556	CE2	TYR	172	-5.829	-4.800	5.740	1.00 15.83	A
ATOM	557	CZ	TYR	172	-5.822	-4.808	7.164	1.00 11.94	A
ATOM	558	OH	TYR	172	-5.995	-6.002	7.820	1.00 12.17	A
ATOM	559	HH	TYR	172	-6.433	-5.843	8.657	1.00 15.00	A
ATOM	560	C	TYR	172	-7.605	-1.276	4.106	1.00 16.85	A
ATOM	561	O	TYR	172	-8.346	-2.057	4.692	1.00 14.06	A
ATOM	562	N	ALA	173	-7.448	-1.141	2.776	1.00 16.29	A
ATOM	563	H	ALA	173	-6.751	-0.490	2.503	1.00 15.00	A
ATOM	564	CA	ALA	173	-7.940	-2.152	1.836	1.00 15.11	A
ATOM	565	CB	ALA	173	-9.300	-1.725	1.292	1.00 12.08	A
ATOM	566	C	ALA	173	-7.007	-2.537	0.653	1.00 15.86	A
ATOM	567	O	ALA	173	-6.147	-1.806	0.191	1.00 14.20	A
ATOM	568	N	GLN	174	-7.244	-3.714	0.109	1.00 16.56	A
ATOM	569	H	GLN	174	-7.774	-4.389	0.620	1.00 15.00	A
ATOM	570	CA	GLN	174	-6.470	-4.119	-1.070	1.00 19.25	A
ATOM	571	CB	GLN	174	-5.582	-5.292	-0.832	1.00 21.99	A
ATOM	572	CG	GLN	174	-4.205	-4.727	-1.030	1.00 30.99	A
ATOM	573	CD	GLN	174	-3.174	-5.845	-0.979	1.00 34.25	A
ATOM	574	OE1	GLN	174	-2.308	-5.899	-0.105	1.00 32.91	A
ATOM	575	NE2	GLN	174	-3.268	-6.699	-2.014	1.00 31.50	A
ATOM	576	HE21	GLN	174	-2.668	-7.487	-1.970	1.00 15.00	A
ATOM	577	HE22	GLN	174	-3.973	-6.621	-2.714	1.00 15.00	A
ATOM	578	C	GLN	174	-7.413	-4.644	-2.114	1.00 19.20	A
ATOM	579	O	GLN	174	-8.285	-5.434	-1.880	1.00 20.03	A
ATOM	580	N	VAL	175	-7.291	-4.107	-3.301	1.00 19.28	A
ATOM	581	H	VAL	175	-6.594	-3.401	-3.400	1.00 15.00	A
ATOM	582	CA	VAL	175	-8.247	-4.500	-4.323	1.00 22.43	A
ATOM	583	CB	VAL	175	-9.319	-3.409	-4.644	1.00 21.41	A
ATOM	584	CG1	VAL	175	-10.146	-2.830	-3.495	1.00 20.17	A
ATOM	585	CG2	VAL	175	-10.268	-4.061	-5.639	1.00 22.88	A
ATOM	586	C	VAL	175	-7.508	-4.859	-5.615	1.00 24.56	A
ATOM	587	O	VAL	175	-6.928	-3.997	-6.301	1.00 23.28	A
ATOM	588	N	THR	176	-7.563	-6.180	-5.879	1.00 25.40	A
ATOM	589	H	THR	176	-7.994	-6.850	-5.250	1.00 15.00	A
ATOM	590	CA	THR	176	-7.086	-6.501	-7.222	1.00 24.46	A
ATOM	591	CB	THR	176	-5.844	-7.454	-7.256	1.00 24.78	A
ATOM	592	OG1	THR	176	-5.948	-8.650	-8.028	1.00 20.31	A
ATOM	593	HG1	THR	176	-5.250	-9.253	-7.796	1.00 15.00	A
ATOM	594	CG2	THR	176	-5.329	-7.711	-5.867	1.00 17.07	A
ATOM	595	C	THR	176	-8.178	-6.700	-8.272	1.00 25.44	A
ATOM	596	O	THR	176	-9.326	-7.043	-7.995	1.00 26.86	A
ATOM	597	N	PHE	177	-7.855	-6.341	-9.506	1.00 22.44	A
ATOM	598	H	PHE	177	-6.920	-6.083	-9.732	1.00 15.00	A
ATOM	599	CA	PHE	177	-8.939	-6.511	-10.479	1.00 22.70	A

ATOM	600	CB	PHE	177	-9.746	-5.194	-10.599	1.00	20.90	A
ATOM	601	CG	PHE	177	-8.813	-4.034	-10.927	1.00	22.51	A
ATOM	602	CD1	PHE	177	-8.771	-3.548	-12.252	1.00	22.11	A
ATOM	603	CD2	PHE	177	-8.011	-3.422	-9.920	1.00	21.87	A
ATOM	604	CE1	PHE	177	-8.041	-2.387	-12.550	1.00	20.53	A
ATOM	605	CE2	PHE	177	-7.289	-2.247	-10.204	1.00	20.44	A
ATOM	606	CZ	PHE	177	-7.376	-1.713	-11.500	1.00	22.79	A
ATOM	607	C	PHE	177	-8.381	-6.949	-11.800	1.00	22.14	A
ATOM	608	O	PHE	177	-7.219	-6.695	-12.072	1.00	21.60	A
ATOM	609	N	CYS	178	-9.210	-7.555	-12.625	1.00	24.52	A
ATOM	610	H	CYS	178	-10.146	-7.797	-12.370	1.00	15.00	A
ATOM	611	CA	CYS	178	-8.599	-7.849	-13.942	1.00	29.77	A
ATOM	612	CB	CYS	178	-8.501	-9.365	-14.214	1.00	32.06	A
ATOM	613	SG	CYS	178	-7.685	-9.731	-15.792	1.00	35.17	A
ATOM	614	C	CYS	178	-9.323	-7.146	-15.088	1.00	28.41	A
ATOM	615	O	CYS	178	-10.534	-7.247	-15.185	1.00	27.54	A
ATOM	616	N	SER	179	-8.589	-6.393	-15.910	1.00	28.86	A
ATOM	617	H	SER	179	-7.608	-6.271	-15.754	1.00	15.00	A
ATOM	618	CA	SER	179	-9.374	-5.454	-16.704	1.00	29.01	A
ATOM	619	CB	SER	179	-9.379	-4.118	-16.020	1.00	30.82	A
ATOM	620	OG	SER	179	-10.615	-3.492	-16.319	1.00	39.79	A
ATOM	621	HG	SER	179	-10.725	-2.812	-15.667	1.00	15.00	A
ATOM	622	C	SER	179	-9.063	-5.196	-18.165	1.00	31.16	A
ATOM	623	O	SER	179	-7.931	-4.953	-18.537	1.00	28.58	A
ATOM	624	N	ASN	180	-10.083	-5.255	-19.042	1.00	35.32	A
ATOM	625	H	ASN	180	-10.966	-5.700	-18.834	1.00	15.00	A
ATOM	626	CA	ASN	180	-9.782	-4.725	-20.366	1.00	34.74	A
ATOM	627	CB	ASN	180	-10.205	-5.554	-21.589	1.00	37.96	A
ATOM	628	CG	ASN	180	-9.650	-4.980	-22.896	1.00	37.12	A
ATOM	629	OD1	ASN	180	-10.058	-3.947	-23.356	1.00	40.66	A
ATOM	630	ND2	ASN	180	-8.619	-5.536	-23.456	1.00	35.85	A
ATOM	631	HD21	ASN	180	-8.343	-6.475	-23.306	1.00	15.00	A
ATOM	632	HD22	ASN	180	-8.153	-4.891	-24.065	1.00	15.00	A
ATOM	633	C	ASN	180	-10.197	-3.331	-20.588	1.00	36.96	A
ATOM	634	O	ASN	180	-11.314	-2.894	-20.433	1.00	37.89	A
ATOM	635	N	ARG	181	-9.147	-2.699	-21.068	1.00	41.95	A
ATOM	636	H	ARG	181	-8.363	-3.318	-21.141	1.00	15.00	A
ATOM	637	CA	ARG	181	-8.997	-1.313	-21.489	1.00	44.24	A
ATOM	638	CB	ARG	181	-7.563	-1.279	-22.026	1.00	43.43	A
ATOM	639	CG	ARG	181	-6.348	-1.638	-21.101	1.00	45.11	A
ATOM	640	CD	ARG	181	-6.235	-2.853	-20.134	1.00	40.68	A
ATOM	641	NE	ARG	181	-5.064	-2.772	-19.271	1.00	46.11	A
ATOM	642	HE	ARG	181	-4.991	-2.058	-18.578	1.00	15.00	A
ATOM	643	CZ	ARG	181	-4.024	-3.611	-19.432	1.00	49.77	A
ATOM	644	NH1	ARG	181	-2.886	-3.414	-18.790	1.00	54.33	A
ATOM	645	HH11	ARG	181	-2.113	-4.032	-18.918	1.00	15.00	A
ATOM	646	HH12	ARG	181	-2.807	-2.642	-18.161	1.00	15.00	A
ATOM	647	NH2	ARG	181	-4.085	-4.641	-20.247	1.00	54.26	A
ATOM	648	HH21	ARG	181	-3.286	-5.230	-20.354	1.00	15.00	A
ATOM	649	HH22	ARG	181	-4.918	-4.833	-20.761	1.00	15.00	A
ATOM	650	C	ARG	181	-10.049	-0.866	-22.499	1.00	47.10	A
ATOM	651	O	ARG	181	-10.979	-0.112	-22.227	1.00	49.20	A
ATOM	652	N	GLU	182	-9.895	-1.447	-23.690	1.00	49.64	A
ATOM	653	H	GLU	182	-9.201	-2.166	-23.775	1.00	15.00	A
ATOM	654	CA	GLU	182	-10.976	-1.385	-24.676	1.00	52.41	A
ATOM	655	CB	GLU	182	-10.437	-2.020	-25.970	1.00	56.93	A
ATOM	656	CG	GLU	182	-10.932	-1.418	-27.295	1.00	66.05	A
ATOM	657	CD	GLU	182	-10.758	0.116	-27.327	1.00	70.54	A
ATOM	658	OE1	GLU	182	-9.613	0.586	-27.442	1.00	72.98	A
ATOM	659	OE2	GLU	182	-11.778	0.830	-27.244	1.00	72.46	A

ATOM	660	C	GLU	182	-12.388	-1.934	-24.304	1.00	53.00	A
ATOM	661	O	GLU	182	-13.379	-1.492	-24.862	1.00	54.27	A
ATOM	662	N	ALA	183	-12.505	-2.877	-23.335	1.00	52.34	A
ATOM	663	H	ALA	183	-11.676	-3.173	-22.865	1.00	15.00	A
ATOM	664	CA	ALA	183	-13.867	-3.258	-22.899	1.00	50.19	A
ATOM	665	CB	ALA	183	-13.855	-4.721	-22.447	1.00	45.02	A
ATOM	666	C	ALA	183	-14.562	-2.321	-21.867	1.00	50.66	A
ATOM	667	O	ALA	183	-15.712	-1.945	-21.990	1.00	47.77	A
ATOM	668	N	SER	184	-13.773	-1.888	-20.878	1.00	52.95	A
ATOM	669	H	SER	184	-12.826	-2.172	-20.991	1.00	15.00	A
ATOM	670	CA	SER	184	-14.228	-1.043	-19.729	1.00	56.78	A
ATOM	671	CB	SER	184	-13.384	-1.397	-18.481	1.00	53.58	A
ATOM	672	OG	SER	184	-13.975	-2.448	-17.721	1.00	47.46	A
ATOM	673	HG	SER	184	-13.291	-3.019	-17.388	1.00	15.00	A
ATOM	674	C	SER	184	-14.183	0.517	-19.880	1.00	59.95	A
ATOM	675	O	SER	184	-13.913	1.297	-18.964	1.00	65.25	A
ATOM	676	N	SER	185	-14.324	0.995	-21.131	1.00	60.08	A
ATOM	677	H	SER	185	-14.623	0.345	-21.831	1.00	15.00	A
ATOM	678	CA	SER	185	-13.825	2.375	-21.391	1.00	60.12	A
ATOM	679	CB	SER	185	-13.522	2.640	-22.869	1.00	60.49	A
ATOM	680	OG	SER	185	-12.243	2.098	-23.242	1.00	59.80	A
ATOM	681	HG	SER	185	-12.158	1.234	-22.833	1.00	15.00	A
ATOM	682	C	SER	185	-14.580	3.589	-20.885	1.00	59.59	A
ATOM	683	O	SER	185	-15.437	4.159	-21.543	1.00	60.08	A
ATOM	684	N	GLN	186	-14.200	3.990	-19.670	1.00	57.71	A
ATOM	685	H	GLN	186	-13.601	3.376	-19.153	1.00	15.00	A
ATOM	686	CA	GLN	186	-15.121	4.936	-18.993	1.00	57.00	A
ATOM	687	CB	GLN	186	-16.094	4.062	-18.175	1.00	58.66	A
ATOM	688	CG	GLN	186	-15.355	3.354	-17.050	1.00	59.69	A
ATOM	689	CD	GLN	186	-16.369	2.789	-16.088	1.00	59.92	A
ATOM	690	OE1	GLN	186	-17.270	3.513	-15.687	1.00	59.81	A
ATOM	691	NE2	GLN	186	-16.249	1.503	-15.787	1.00	59.63	A
ATOM	692	HE21	GLN	186	-15.492	0.948	-16.113	1.00	15.00	A
ATOM	693	HE22	GLN	186	-16.950	1.119	-15.168	1.00	15.00	A
ATOM	694	C	GLN	186	-14.758	6.290	-18.221	1.00	54.36	A
ATOM	695	O	GLN	186	-15.596	7.198	-18.298	1.00	53.98	A
ATOM	696	N	ALA	187	-13.566	6.424	-17.511	1.00	50.35	A
ATOM	697	H	ALA	187	-13.476	7.274	-16.970	1.00	15.00	A
ATOM	698	CA	ALA	187	-12.388	5.599	-17.832	1.00	43.26	A
ATOM	699	CB	ALA	187	-11.546	6.284	-18.918	1.00	38.95	A
ATOM	700	C	ALA	187	-11.456	4.882	-16.849	1.00	40.48	A
ATOM	701	O	ALA	187	-10.887	3.875	-17.295	1.00	43.24	A
ATOM	702	N	PRO	188	-11.210	5.383	-15.594	1.00	38.66	A
ATOM	703	CD	PRO	188	-11.543	6.687	-15.000	1.00	38.15	A
ATOM	704	CA	PRO	188	-10.220	4.665	-14.751	1.00	35.94	A
ATOM	705	CB	PRO	188	-9.395	5.813	-14.150	1.00	33.99	A
ATOM	706	CG	PRO	188	-10.377	7.000	-14.036	1.00	32.69	A
ATOM	707	C	PRO	188	-10.840	3.783	-13.683	1.00	33.66	A
ATOM	708	O	PRO	188	-11.885	4.062	-13.140	1.00	33.41	A
ATOM	709	N	PHE	189	-10.147	2.695	-13.346	1.00	28.66	A
ATOM	710	H	PHE	189	-9.260	2.508	-13.748	1.00	15.00	A
ATOM	711	CA	PHE	189	-10.721	2.013	-12.171	1.00	26.71	A
ATOM	712	CB	PHE	189	-10.122	0.601	-12.034	1.00	26.21	A
ATOM	713	CG	PHE	189	-10.671	-0.189	-10.849	1.00	22.92	A
ATOM	714	CD1	PHE	189	-10.126	0.005	-9.566	1.00	17.72	A
ATOM	715	CD2	PHE	189	-11.687	-1.165	-11.064	1.00	21.88	A
ATOM	716	CE1	PHE	189	-10.590	-0.815	-8.522	1.00	19.12	A
ATOM	717	CE2	PHE	189	-12.124	-1.995	-10.011	1.00	21.13	A
ATOM	718	CZ	PHE	189	-11.571	-1.806	-8.736	1.00	18.44	A
ATOM	719	C	PHE	189	-10.445	2.815	-10.909	1.00	27.14	A

ATOM	720	O	PHE	189	-9.308	3.244	-10.706	1.00 28.72	A
ATOM	721	N	ILE	190	-11.468	2.964	-10.071	1.00 24.71	A
ATOM	722	H	ILE	190	-12.408	2.786	-10.388	1.00 15.00	A
ATOM	723	CA	ILE	190	-11.193	3.626	-8.788	1.00 24.03	A
ATOM	724	CB	ILE	190	-11.316	5.242	-8.743	1.00 26.86	A
ATOM	725	CG2	ILE	190	-11.892	5.979	-9.997	1.00 19.87	A
ATOM	726	CG1	ILE	190	-11.801	5.888	-7.424	1.00 22.54	A
ATOM	727	CD1	ILE	190	-12.819	7.012	-7.645	1.00 28.56	A
ATOM	728	C	ILE	190	-11.844	2.812	-7.656	1.00 21.97	A
ATOM	729	O	ILE	190	-12.891	2.197	-7.801	1.00 16.30	A
ATOM	730	N	ALA	191	-11.026	2.700	-6.590	1.00 17.21	A
ATOM	731	H	ALA	191	-10.124	3.124	-6.662	1.00 15.00	A
ATOM	732	CA	ALA	191	-11.501	2.195	-5.321	1.00 15.20	A
ATOM	733	CB	ALA	191	-10.730	0.928	-4.968	1.00 14.79	A
ATOM	734	C	ALA	191	-11.439	3.230	-4.206	1.00 17.11	A
ATOM	735	O	ALA	191	-10.467	3.961	-4.052	1.00 14.04	A
ATOM	736	N	SER	192	-12.511	3.245	-3.433	1.00 14.72	A
ATOM	737	H	SER	192	-13.277	2.694	-3.804	1.00 15.00	A
ATOM	738	CA	SER	192	-12.725	4.289	-2.423	1.00 16.69	A
ATOM	739	CB	SER	192	-13.931	5.144	-2.803	1.00 14.83	A
ATOM	740	OG	SER	192	-13.556	5.828	-3.994	1.00 21.23	A
ATOM	741	HG	SER	192	-14.367	5.966	-4.520	1.00 15.00	A
ATOM	742	C	SER	192	-12.980	3.682	-1.069	1.00 17.77	A
ATOM	743	O	SER	192	-13.753	2.738	-0.947	1.00 20.76	A
ATOM	744	N	LEU	193	-12.285	4.209	-0.038	1.00 15.56	A
ATOM	745	H	LEU	193	-11.681	4.959	-0.280	1.00 15.00	A
ATOM	746	CA	LEU	193	-12.510	3.761	1.366	1.00 13.27	A
ATOM	747	CB	LEU	193	-11.195	3.825	2.217	1.00 12.74	A
ATOM	748	CG	LEU	193	-11.051	3.141	3.604	1.00 14.37	A
ATOM	749	CD1	LEU	193	-12.272	2.354	4.116	1.00 14.67	A
ATOM	750	CD2	LEU	193	-10.274	3.986	4.622	1.00 12.64	A
ATOM	751	C	LEU	193	-13.497	4.748	1.911	1.00 11.22	A
ATOM	752	O	LEU	193	-13.188	5.912	1.903	1.00 12.22	A
ATOM	753	N	CYS	194	-14.652	4.326	2.310	1.00 13.66	A
ATOM	754	H	CYS	194	-14.828	3.347	2.276	1.00 15.00	A
ATOM	755	CA	CYS	194	-15.595	5.360	2.713	1.00 14.84	A
ATOM	756	CB	CYS	194	-16.915	5.409	1.918	1.00 17.58	A
ATOM	757	SG	CYS	194	-16.623	5.417	0.165	1.00 16.33	A
ATOM	758	C	CYS	194	-16.046	5.163	4.137	1.00 12.81	A
ATOM	759	O	CYS	194	-15.983	4.072	4.655	1.00 10.34	A
ATOM	760	N	LEU	195	-16.557	6.254	4.697	1.00 14.32	A
ATOM	761	H	LEU	195	-16.541	7.088	4.154	1.00 15.00	A
ATOM	762	CA	LEU	195	-17.039	6.291	6.076	1.00 14.89	A
ATOM	763	CB	LEU	195	-16.195	7.372	6.789	1.00 15.56	A
ATOM	764	CG	LEU	195	-16.571	7.680	8.242	1.00 15.56	A
ATOM	765	CD1	LEU	195	-15.932	8.967	8.762	1.00 13.72	A
ATOM	766	CD2	LEU	195	-16.463	6.448	9.154	1.00 17.25	A
ATOM	767	C	LEU	195	-18.546	6.544	6.209	1.00 13.54	A
ATOM	768	O	LEU	195	-19.038	7.521	5.705	1.00 14.56	A
ATOM	769	N	LYS	196	-19.238	5.667	6.905	1.00 16.36	A
ATOM	770	H	LYS	196	-18.719	4.875	7.197	1.00 15.00	A
ATOM	771	CA	LYS	196	-20.577	5.972	7.405	1.00 21.01	A
ATOM	772	CB	LYS	196	-21.475	4.726	7.146	1.00 22.66	A
ATOM	773	CG	LYS	196	-22.953	4.839	7.590	1.00 31.25	A
ATOM	774	CD	LYS	196	-23.364	4.915	9.104	1.00 40.25	A
ATOM	775	CE	LYS	196	-23.189	3.694	10.060	1.00 43.56	A
ATOM	776	NZ	LYS	196	-23.004	4.158	11.453	1.00 44.46	A
ATOM	777	HZ1	LYS	196	-22.182	4.799	11.467	1.00 15.00	A
ATOM	778	HZ2	LYS	196	-23.847	4.665	11.778	1.00 15.00	A
ATOM	779	HZ3	LYS	196	-22.807	3.334	12.066	1.00 15.00	A

ATOM	780	C	LYS	196	-20.478	6.290	8.899	1.00 19.25	A
ATOM	781	O	LYS	196	-20.194	5.434	9.714	1.00 18.35	A
ATOM	782	N	SER	197	-20.664	7.534	9.272	1.00 20.63	A
ATOM	783	H	SER	197	-20.891	8.247	8.615	1.00 15.00	A
ATOM	784	CA	SER	197	-20.752	7.701	10.729	1.00 24.87	A
ATOM	785	CB	SER	197	-19.898	8.878	11.207	1.00 25.62	A
ATOM	786	OG	SER	197	-19.563	8.687	12.588	1.00 32.22	A
ATOM	787	HG	SER	197	-18.795	8.110	12.611	1.00 15.00	A
ATOM	788	C	SER	197	-22.216	7.810	11.218	1.00 26.33	A
ATOM	789	O	SER	197	-23.078	8.303	10.497	1.00 26.57	A
ATOM	790	N	PRO	198	-22.534	7.274	12.407	1.00 26.77	A
ATOM	791	CD	PRO	198	-21.649	6.526	13.301	1.00 32.92	A
ATOM	792	CA	PRO	198	-23.919	7.381	12.913	1.00 28.73	A
ATOM	793	CB	PRO	198	-23.784	6.789	14.318	1.00 32.89	A
ATOM	794	CG	PRO	198	-22.289	6.726	14.659	1.00 33.55	A
ATOM	795	C	PRO	198	-24.591	8.789	12.847	1.00 26.60	A
ATOM	796	O	PRO	198	-24.035	9.817	13.242	1.00 20.20	A
ATOM	797	N	GLY	199	-25.729	8.773	12.119	1.00 25.75	A
ATOM	798	H	GLY	199	-26.170	7.857	12.057	1.00 15.00	A
ATOM	799	CA	GLY	199	-26.486	10.003	11.790	1.00 26.91	A
ATOM	800	C	GLY	199	-25.821	10.971	10.816	1.00 28.98	A
ATOM	801	O	GLY	199	-26.084	12.151	10.797	1.00 31.05	A
ATOM	802	N	ARG	200	-24.898	10.464	10.001	1.00 30.15	A
ATOM	803	H	ARG	200	-24.629	9.519	10.165	1.00 15.00	A
ATOM	804	CA	ARG	200	-24.140	11.384	9.166	1.00 28.98	A
ATOM	805	CB	ARG	200	-22.749	11.590	9.783	1.00 33.16	A
ATOM	806	CG	ARG	200	-22.739	12.290	11.162	1.00 38.34	A
ATOM	807	CD	ARG	200	-21.327	12.530	11.705	1.00 42.14	A
ATOM	808	NE	ARG	200	-21.292	12.875	13.131	1.00 43.64	A
ATOM	809	HE	ARG	200	-21.327	13.831	13.424	1.00 15.00	A
ATOM	810	CZ	ARG	200	-21.138	11.896	14.051	1.00 46.40	A
ATOM	811	NH1	ARG	200	-21.219	10.603	13.733	1.00 46.31	A
ATOM	812	HH11	ARG	200	-21.104	9.910	14.445	1.00 15.00	A
ATOM	813	HH12	ARG	200	-21.394	10.320	12.789	1.00 15.00	A
ATOM	814	NH2	ARG	200	-20.901	12.226	15.311	1.00 46.65	A
ATOM	815	HH21	ARG	200	-20.847	13.193	15.566	1.00 15.00	A
ATOM	816	HH22	ARG	200	-20.785	11.510	16.002	1.00 15.00	A
ATOM	817	C	ARG	200	-24.084	10.967	7.710	1.00 27.77	A
ATOM	818	O	ARG	200	-24.264	9.791	7.449	1.00 28.21	A
ATOM	819	N	PHE	201	-23.853	11.926	6.792	1.00 30.83	A
ATOM	820	H	PHE	201	-23.513	12.821	7.126	1.00 15.00	A
ATOM	821	CA	PHE	201	-24.016	11.708	5.339	1.00 34.17	A
ATOM	822	CB	PHE	201	-23.851	12.996	4.572	1.00 31.58	A
ATOM	823	CG	PHE	201	-25.154	13.730	4.614	1.00 34.85	A
ATOM	824	CD1	PHE	201	-25.174	15.062	5.081	1.00 37.56	A
ATOM	825	CD2	PHE	201	-26.335	13.081	4.190	1.00 37.89	A
ATOM	826	CE1	PHE	201	-26.397	15.749	5.182	1.00 36.91	A
ATOM	827	CE2	PHE	201	-27.566	13.762	4.280	1.00 38.98	A
ATOM	828	CZ	PHE	201	-27.572	15.065	4.815	1.00 37.61	A
ATOM	829	C	PHE	201	-23.277	10.605	4.545	1.00 39.40	A
ATOM	830	O	PHE	201	-23.853	10.034	3.604	1.00 45.71	A
ATOM	831	N	GLU	202	-22.031	10.316	5.034	1.00 35.75	A
ATOM	832	H	GLU	202	-21.878	10.753	5.925	1.00 15.00	A
ATOM	833	CA	GLU	202	-20.964	9.564	4.318	1.00 34.52	A
ATOM	834	CB	GLU	202	-21.295	8.540	3.234	1.00 33.66	A
ATOM	835	CG	GLU	202	-21.924	7.245	3.713	1.00 40.61	A
ATOM	836	CD	GLU	202	-22.647	6.505	2.561	1.00 46.12	A
ATOM	837	OE1	GLU	202	-23.461	5.613	2.886	1.00 46.89	A
ATOM	838	OE2	GLU	202	-22.417	6.814	1.370	1.00 45.63	A
ATOM	839	C	GLU	202	-19.924	10.450	3.717	1.00 29.99	A

ATOM	840	O	GLU	202	-20.137	11.567	3.300	1.00	30.76	A
ATOM	841	N	ARG	203	-18.728	9.897	3.856	1.00	26.88	A
ATOM	842	H	ARG	203	-18.690	8.998	4.285	1.00	15.00	A
ATOM	843	CA	ARG	203	-17.539	10.603	3.358	1.00	21.88	A
ATOM	844	CB	ARG	203	-16.819	11.410	4.457	1.00	27.07	A
ATOM	845	CG	ARG	203	-17.681	12.187	5.467	1.00	37.32	A
ATOM	846	CD	ARG	203	-16.894	13.213	6.339	1.00	48.09	A
ATOM	847	NE	ARG	203	-15.911	12.667	7.308	1.00	56.90	A
ATOM	848	HE	ARG	203	-16.240	12.433	8.223	1.00	15.00	A
ATOM	849	CZ	ARG	203	-14.572	12.475	7.001	1.00	66.77	A
ATOM	850	NH1	ARG	203	-13.702	12.002	7.911	1.00	68.44	A
ATOM	851	HH11	ARG	203	-12.745	11.829	7.666	1.00	15.00	A
ATOM	852	HH12	ARG	203	-14.016	11.822	8.845	1.00	15.00	A
ATOM	853	NH2	ARG	203	-14.084	12.716	5.766	1.00	67.68	A
ATOM	854	HH21	ARG	203	-14.670	13.108	5.060	1.00	15.00	A
ATOM	855	HH22	ARG	203	-13.143	12.499	5.544	1.00	15.00	A
ATOM	856	C	ARG	203	-16.517	9.633	2.678	1.00	17.71	A
ATOM	857	O	ARG	203	-16.375	8.418	2.931	1.00	7.69	A
ATOM	858	N	ILE	204	-15.789	10.253	1.791	1.00	14.42	A
ATOM	859	H	ILE	204	-15.915	11.228	1.561	1.00	15.00	A
ATOM	860	CA	ILE	204	-14.662	9.482	1.353	1.00	18.32	A
ATOM	861	CB	ILE	204	-14.520	9.392	-0.231	1.00	24.52	A
ATOM	862	CG2	ILE	204	-15.820	9.529	-1.069	1.00	21.85	A
ATOM	863	CG1	ILE	204	-13.439	10.195	-0.949	1.00	26.35	A
ATOM	864	CD1	ILE	204	-13.992	11.231	-1.961	1.00	36.33	A
ATOM	865	C	ILE	204	-13.387	9.819	2.153	1.00	16.58	A
ATOM	866	O	ILE	204	-13.070	10.956	2.457	1.00	18.63	A
ATOM	867	N	LEU	205	-12.718	8.725	2.571	1.00	13.32	A
ATOM	868	H	LEU	205	-13.142	7.853	2.321	1.00	15.00	A
ATOM	869	CA	LEU	205	-11.467	8.829	3.322	1.00	10.01	A
ATOM	870	CB	LEU	205	-11.440	7.688	4.382	1.00	6.66	A
ATOM	871	CG	LEU	205	-12.571	7.727	5.441	1.00	7.99	A
ATOM	872	CD1	LEU	205	-12.722	9.088	6.089	1.00	8.78	A
ATOM	873	CD2	LEU	205	-12.419	6.720	6.582	1.00	8.08	A
ATOM	874	C	LEU	205	-10.268	8.811	2.377	1.00	9.75	A
ATOM	875	O	LEU	205	-9.416	9.655	2.320	1.00	10.25	A
ATOM	876	N	LEU	206	-10.252	7.769	1.562	1.00	10.28	A
ATOM	877	H	LEU	206	-10.991	7.119	1.684	1.00	15.00	A
ATOM	878	CA	LEU	206	-9.166	7.555	0.610	1.00	10.02	A
ATOM	879	CB	LEU	206	-8.249	6.384	0.990	1.00	11.94	A
ATOM	880	CG	LEU	206	-7.001	6.527	1.859	1.00	14.40	A
ATOM	881	CD1	LEU	206	-7.094	5.595	3.074	1.00	14.49	A
ATOM	882	CD2	LEU	206	-6.531	7.958	2.151	1.00	8.78	A
ATOM	883	C	LEU	206	-9.756	7.071	-0.697	1.00	11.91	A
ATOM	884	O	LEU	206	-10.792	6.406	-0.778	1.00	10.67	A
ATOM	885	N	ARG	207	-9.005	7.428	-1.720	1.00	8.06	A
ATOM	886	H	ARG	207	-8.196	7.992	-1.553	1.00	15.00	A
ATOM	887	CA	ARG	207	-9.309	6.823	-2.992	1.00	10.45	A
ATOM	888	CB	ARG	207	-9.974	7.790	-3.904	1.00	8.71	A
ATOM	889	CG	ARG	207	-11.258	8.270	-3.357	1.00	15.68	A
ATOM	890	CD	ARG	207	-11.652	9.459	-4.163	1.00	22.25	A
ATOM	891	NE	ARG	207	-12.670	9.192	-5.171	1.00	29.59	A
ATOM	892	HE	ARG	207	-13.115	8.300	-5.249	1.00	15.00	A
ATOM	893	CZ	ARG	207	-13.063	10.272	-5.919	1.00	40.09	A
ATOM	894	NH1	ARG	207	-12.482	11.498	-5.813	1.00	36.32	A
ATOM	895	HH11	ARG	207	-12.813	12.246	-6.391	1.00	15.00	A
ATOM	896	HH12	ARG	207	-11.737	11.651	-5.165	1.00	15.00	A
ATOM	897	NH2	ARG	207	-14.067	10.111	-6.773	1.00	40.86	A
ATOM	898	HH21	ARG	207	-14.392	10.877	-7.329	1.00	15.00	A
ATOM	899	HH22	ARG	207	-14.498	9.207	-6.853	1.00	15.00	A

ATOM	900	C	ARG	207	-8.044	6.456	-3.741	1.00 12.59	A
ATOM	901	O	ARG	207	-7.053	7.150	-3.787	1.00 15.58	A
ATOM	902	N	ALA	208	-8.096	5.358	-4.465	1.00 17.06	A
ATOM	903	H	ALA	208	-8.879	4.758	-4.355	1.00 15.00	A
ATOM	904	CA	ALA	208	-7.025	5.128	-5.465	1.00 17.00	A
ATOM	905	CB	ALA	208	-6.052	4.020	-5.072	1.00 14.69	A
ATOM	906	C	ALA	208	-7.544	4.830	-6.854	1.00 20.46	A
ATOM	907	O	ALA	208	-8.438	4.020	-7.057	1.00 21.89	A
ATOM	908	N	ALA	209	-6.986	5.586	-7.808	1.00 26.22	A
ATOM	909	H	ALA	209	-6.280	6.235	-7.533	1.00 15.00	A
ATOM	910	CA	ALA	209	-7.253	5.208	-9.196	1.00 28.06	A
ATOM	911	CB	ALA	209	-7.702	6.380	-10.069	1.00 27.10	A
ATOM	912	C	ALA	209	-6.075	4.461	-9.832	1.00 32.54	A
ATOM	913	O	ALA	209	-4.895	4.726	-9.593	1.00 33.00	A
ATOM	914	N	ASN	210	-6.502	3.491	-10.634	1.00 32.11	A
ATOM	915	H	ASN	210	-7.466	3.249	-10.531	1.00 15.00	A
ATOM	916	CA	ASN	210	-5.674	2.893	-11.662	1.00 36.00	A
ATOM	917	CB	ASN	210	-5.366	1.446	-11.355	1.00 39.53	A
ATOM	918	CG	ASN	210	-4.463	1.366	-10.154	1.00 42.59	A
ATOM	919	OD1	ASN	210	-4.285	2.273	-9.342	1.00 39.26	A
ATOM	920	ND2	ASN	210	-3.951	0.165	-10.055	1.00 41.77	A
ATOM	921	HD21	ASN	210	-3.990	-0.479	-10.817	1.00 15.00	A
ATOM	922	HD22	ASN	210	-3.364	-0.081	-9.279	1.00 15.00	A
ATOM	923	C	ASN	210	-6.299	2.931	-13.043	1.00 36.95	A
ATOM	924	O	ASN	210	-7.492	2.752	-13.259	1.00 36.93	A
ATOM	925	N	THR	211	-5.447	3.168	-14.013	1.00 37.83	A
ATOM	926	H	THR	211	-4.484	3.377	-13.821	1.00 15.00	A
ATOM	927	CA	THR	211	-6.119	3.224	-15.314	1.00 41.27	A
ATOM	928	CB	THR	211	-5.325	4.158	-16.268	1.00 44.53	A
ATOM	929	OG1	THR	211	-6.076	4.506	-17.438	1.00 49.34	A
ATOM	930	HG1	THR	211	-6.032	5.493	-17.508	1.00 15.00	A
ATOM	931	CG2	THR	211	-3.926	3.604	-16.581	1.00 46.08	A
ATOM	932	C	THR	211	-6.434	1.833	-15.878	1.00 39.17	A
ATOM	933	O	THR	211	-5.822	0.863	-15.475	1.00 36.48	A
ATOM	934	N	HIS	212	-7.416	1.718	-16.789	1.00 37.14	A
ATOM	935	H	HIS	212	-8.106	2.438	-16.878	1.00 15.00	A
ATOM	936	CA	HIS	212	-7.294	0.454	-17.529	1.00 33.23	A
ATOM	937	CB	HIS	212	-8.680	-0.012	-18.082	1.00 27.73	A
ATOM	938	CG	HIS	212	-9.856	0.060	-17.111	1.00 24.58	A
ATOM	939	ND1	HIS	212	-10.862	0.967	-17.161	1.00 24.59	A
ATOM	940	HD1	HIS	212	-11.000	1.702	-17.794	1.00 15.00	A
ATOM	941	CD2	HIS	212	-10.049	-0.723	-15.985	1.00 20.65	A
ATOM	942	NE2	HIS	212	-11.154	-0.265	-15.383	1.00 24.01	A
ATOM	943	CE1	HIS	212	-11.665	0.780	-16.092	1.00 17.59	A
ATOM	944	C	HIS	212	-6.257	0.633	-18.683	1.00 38.31	A
ATOM	945	O	HIS	212	-5.363	-0.132	-18.923	1.00 33.92	A
ATOM	946	N	SER	213	-6.444	1.737	-19.443	1.00 46.63	A
ATOM	947	H	SER	213	-7.156	2.323	-19.055	1.00 15.00	A
ATOM	948	CA	SER	213	-5.705	2.177	-20.675	1.00 53.91	A
ATOM	949	CB	SER	213	-4.272	2.704	-20.400	1.00 52.61	A
ATOM	950	OG	SER	213	-3.266	1.697	-20.547	1.00 53.97	A
ATOM	951	HG	SER	213	-3.363	1.064	-19.823	1.00 15.00	A
ATOM	952	C	SER	213	-5.844	1.508	-22.097	1.00 60.03	A
ATOM	953	O	SER	213	-5.005	0.811	-22.682	1.00 61.19	A
ATOM	954	N	SER	214	-7.043	1.803	-22.686	1.00 64.96	A
ATOM	955	H	SER	214	-7.705	2.322	-22.146	1.00 15.00	A
ATOM	956	CA	SER	214	-7.463	1.456	-24.094	1.00 69.62	A
ATOM	957	CB	SER	214	-8.727	2.218	-24.495	1.00 67.82	A
ATOM	958	OG	SER	214	-9.563	2.257	-23.336	1.00 67.64	A
ATOM	959	HG	SER	214	-10.468	2.398	-23.623	1.00 15.00	A

ATOM	960	C	SER	214	-6.518	1.587	-25.300	1.00	72.08	A
ATOM	961	O	SER	214	-6.102	2.683	-25.686	1.00	73.45	A
ATOM	962	N	ALA	215	-6.175	0.409	-25.899	1.00	73.38	A
ATOM	963	H	ALA	215	-5.456	0.596	-26.565	1.00	15.00	A
ATOM	964	CA	ALA	215	-6.858	-0.915	-25.753	1.00	72.62	A
ATOM	965	CB	ALA	215	-7.199	-1.505	-27.138	1.00	73.08	A
ATOM	966	C	ALA	215	-6.331	-2.148	-24.983	1.00	72.11	A
ATOM	967	O	ALA	215	-7.020	-3.161	-25.069	1.00	72.74	A
ATOM	968	N	LYS	216	-5.153	-2.076	-24.282	1.00	70.17	A
ATOM	969	H	LYS	216	-4.747	-1.165	-24.199	1.00	15.00	A
ATOM	970	CA	LYS	216	-4.482	-3.256	-23.626	1.00	67.38	A
ATOM	971	CB	LYS	216	-3.458	-2.691	-22.648	1.00	65.30	A
ATOM	972	CG	LYS	216	-2.217	-2.107	-23.321	1.00	66.86	A
ATOM	973	CD	LYS	216	-1.419	-3.149	-24.134	1.00	68.81	A
ATOM	974	CE	LYS	216	-0.082	-2.674	-24.740	1.00	67.51	A
ATOM	975	NZ	LYS	216	0.483	-3.722	-25.598	1.00	67.80	A
ATOM	976	HZ1	LYS	216	0.620	-4.590	-25.041	1.00	15.00	A
ATOM	977	HZ2	LYS	216	-0.168	-3.914	-26.385	1.00	15.00	A
ATOM	978	HZ3	LYS	216	1.401	-3.406	-25.973	1.00	15.00	A
ATOM	979	C	LYS	216	-5.321	-4.441	-22.993	1.00	66.99	A
ATOM	980	O	LYS	216	-6.462	-4.266	-22.575	1.00	69.90	A
ATOM	981	N	PRO	217	-4.835	-5.724	-22.952	1.00	65.06	A
ATOM	982	CD	PRO	217	-3.525	-6.262	-23.308	1.00	67.91	A
ATOM	983	CA	PRO	217	-5.792	-6.827	-22.626	1.00	62.80	A
ATOM	984	CB	PRO	217	-5.285	-8.004	-23.464	1.00	64.33	A
ATOM	985	CG	PRO	217	-3.755	-7.799	-23.338	1.00	69.63	A
ATOM	986	C	PRO	217	-5.837	-7.237	-21.150	1.00	59.77	A
ATOM	987	O	PRO	217	-4.747	-7.318	-20.589	1.00	58.81	A
ATOM	988	N	CYS	218	-7.115	-7.516	-20.627	1.00	55.45	A
ATOM	989	H	CYS	218	-7.874	-7.287	-21.233	1.00	15.00	A
ATOM	990	CA	CYS	218	-7.433	-7.929	-19.210	1.00	46.55	A
ATOM	991	CB	CYS	218	-8.105	-9.289	-19.079	1.00	44.69	A
ATOM	992	SG	CYS	218	-8.855	-9.822	-17.460	1.00	43.11	A
ATOM	993	C	CYS	218	-6.265	-7.994	-18.263	1.00	43.24	A
ATOM	994	O	CYS	218	-5.720	-9.026	-17.959	1.00	44.68	A
ATOM	995	N	GLY	219	-5.853	-6.820	-17.876	1.00	40.28	A
ATOM	996	H	GLY	219	-6.328	-5.961	-18.059	1.00	15.00	A
ATOM	997	CA	GLY	219	-4.659	-6.828	-17.070	1.00	36.27	A
ATOM	998	C	GLY	219	-5.017	-7.080	-15.643	1.00	33.86	A
ATOM	999	O	GLY	219	-5.906	-6.452	-15.097	1.00	34.90	A
ATOM	1000	N	GLN	220	-4.313	-7.996	-15.023	1.00	33.15	A
ATOM	1001	H	GLN	220	-3.835	-8.684	-15.580	1.00	15.00	A
ATOM	1002	CA	GLN	220	-4.448	-7.929	-13.578	1.00	29.92	A
ATOM	1003	CB	GLN	220	-4.298	-9.282	-12.936	1.00	27.81	A
ATOM	1004	CG	GLN	220	-5.380	-9.340	-11.883	1.00	30.94	A
ATOM	1005	CD	GLN	220	-5.285	-10.631	-11.132	1.00	36.37	A
ATOM	1006	OE1	GLN	220	-4.216	-10.969	-10.661	1.00	38.47	A
ATOM	1007	NE2	GLN	220	-6.425	-11.296	-10.977	1.00	37.61	A
ATOM	1008	HE21	GLN	220	-6.295	-12.235	-10.667	1.00	15.00	A
ATOM	1009	HE22	GLN	220	-7.373	-11.036	-11.200	1.00	15.00	A
ATOM	1010	C	GLN	220	-3.666	-6.845	-12.859	1.00	27.48	A
ATOM	1011	O	GLN	220	-2.461	-6.694	-12.999	1.00	27.61	A
ATOM	1012	N	GLN	221	-4.438	-6.040	-12.110	1.00	25.10	A
ATOM	1013	H	GLN	221	-5.433	-6.174	-12.143	1.00	15.00	A
ATOM	1014	CA	GLN	221	-3.803	-4.929	-11.387	1.00	22.41	A
ATOM	1015	CB	GLN	221	-4.077	-3.528	-11.949	1.00	22.12	A
ATOM	1016	CG	GLN	221	-3.284	-3.029	-13.163	1.00	32.16	A
ATOM	1017	CD	GLN	221	-3.795	-1.637	-13.405	1.00	34.69	A
ATOM	1018	OE1	GLN	221	-3.746	-0.763	-12.558	1.00	42.12	A
ATOM	1019	NE2	GLN	221	-4.648	-1.507	-14.398	1.00	34.93	A

ATOM	1020	HE21	GLN	221	-4.981	-2.187	-15.042	1.00	15.00	A
ATOM	1021	HE22	GLN	221	-4.844	-0.551	-14.575	1.00	15.00	A
ATOM	1022	C	GLN	221	-4.227	-4.913	-9.948	1.00	19.54	A
ATOM	1023	O	GLN	221	-5.300	-5.381	-9.611	1.00	19.46	A
ATOM	1024	N	SER	222	-3.374	-4.330	-9.123	1.00	18.12	A
ATOM	1025	H	SER	222	-2.442	-4.098	-9.441	1.00	15.00	A
ATOM	1026	CA	SER	222	-3.851	-4.120	-7.752	1.00	19.45	A
ATOM	1027	CB	SER	222	-3.104	-4.947	-6.691	1.00	19.99	A
ATOM	1028	OG	SER	222	-3.096	-6.339	-7.053	1.00	24.64	A
ATOM	1029	HG	SER	222	-2.651	-6.336	-7.904	1.00	15.00	A
ATOM	1030	C	SER	222	-3.731	-2.688	-7.330	1.00	24.09	A
ATOM	1031	O	SER	222	-2.992	-1.929	-7.944	1.00	29.41	A
ATOM	1032	N	ILE	223	-4.534	-2.386	-6.283	1.00	22.81	A
ATOM	1033	H	ILE	223	-5.172	-3.127	-6.074	1.00	15.00	A
ATOM	1034	CA	ILE	223	-4.567	-1.122	-5.530	1.00	21.06	A
ATOM	1035	CB	ILE	223	-5.970	-0.490	-5.852	1.00	19.87	A
ATOM	1036	CG2	ILE	223	-6.564	0.315	-4.673	1.00	16.59	A
ATOM	1037	CG1	ILE	223	-5.911	0.278	-7.188	1.00	15.22	A
ATOM	1038	CD1	ILE	223	-7.229	0.868	-7.709	1.00	20.54	A
ATOM	1039	C	ILE	223	-4.367	-1.446	-4.007	1.00	21.62	A
ATOM	1040	O	ILE	223	-5.098	-2.269	-3.444	1.00	19.58	A
ATOM	1041	N	HIS	224	-3.429	-0.767	-3.340	1.00	19.73	A
ATOM	1042	H	HIS	224	-2.794	-0.230	-3.899	1.00	15.00	A
ATOM	1043	CA	HIS	224	-3.497	-0.671	-1.858	1.00	16.45	A
ATOM	1044	CB	HIS	224	-2.164	-1.183	-1.227	1.00	18.74	A
ATOM	1045	CG	HIS	224	-2.182	-1.442	0.296	1.00	14.92	A
ATOM	1046	ND1	HIS	224	-2.479	-2.628	0.882	1.00	15.33	A
ATOM	1047	HD1	HIS	224	-2.667	-3.515	0.505	1.00	15.00	A
ATOM	1048	CD2	HIS	224	-1.964	-0.524	1.310	1.00	13.79	A
ATOM	1049	NE2	HIS	224	-2.137	-1.127	2.517	1.00	10.52	A
ATOM	1050	CE1	HIS	224	-2.458	-2.411	2.232	1.00	11.70	A
ATOM	1051	C	HIS	224	-3.914	0.699	-1.284	1.00	15.18	A
ATOM	1052	O	HIS	224	-3.338	1.732	-1.520	1.00	14.36	A
ATOM	1053	N	LEU	225	-4.970	0.673	-0.468	1.00	16.85	A
ATOM	1054	H	LEU	225	-5.317	-0.238	-0.252	1.00	15.00	A
ATOM	1055	CA	LEU	225	-5.395	1.885	0.256	1.00	15.55	A
ATOM	1056	CB	LEU	225	-6.927	2.082	0.208	1.00	17.15	A
ATOM	1057	CG	LEU	225	-7.495	2.456	-1.154	1.00	18.03	A
ATOM	1058	CD1	LEU	225	-6.792	3.659	-1.774	1.00	19.34	A
ATOM	1059	CD2	LEU	225	-8.994	2.659	-1.098	1.00	13.66	A
ATOM	1060	C	LEU	225	-5.074	1.758	1.739	1.00	14.77	A
ATOM	1061	O	LEU	225	-5.347	0.726	2.345	1.00	12.20	A
ATOM	1062	N	GLY	226	-4.544	2.829	2.344	1.00	18.04	A
ATOM	1063	H	GLY	226	-4.218	3.616	1.813	1.00	15.00	A
ATOM	1064	CA	GLY	226	-4.541	2.833	3.841	1.00	18.37	A
ATOM	1065	C	GLY	226	-4.193	4.171	4.544	1.00	17.08	A
ATOM	1066	O	GLY	226	-3.389	4.906	4.055	1.00	13.75	A
ATOM	1067	N	GLY	227	-4.781	4.457	5.725	1.00	16.30	A
ATOM	1068	H	GLY	227	-5.434	3.771	6.036	1.00	15.00	A
ATOM	1069	CA	GLY	227	-4.379	5.649	6.490	1.00	8.52	A
ATOM	1070	C	GLY	227	-4.935	5.631	7.959	1.00	12.75	A
ATOM	1071	O	GLY	227	-5.651	4.748	8.466	1.00	10.57	A
ATOM	1072	N	VAL	228	-4.588	6.698	8.675	1.00	9.23	A
ATOM	1073	H	VAL	228	-4.040	7.398	8.222	1.00	15.00	A
ATOM	1074	CA	VAL	228	-5.110	6.818	10.067	1.00	11.74	A
ATOM	1075	CB	VAL	228	-4.085	7.320	11.144	1.00	14.30	A
ATOM	1076	CG1	VAL	228	-2.830	6.445	11.333	1.00	10.73	A
ATOM	1077	CG2	VAL	228	-4.789	7.565	12.479	1.00	17.07	A
ATOM	1078	C	VAL	228	-6.238	7.803	10.098	1.00	9.03	A
ATOM	1079	O	VAL	228	-6.089	8.937	9.649	1.00	12.01	A

ATOM	1080	N	PHE	229	-7.347	7.299	10.640	1.00	9.88	A
ATOM	1081	H	PHE	229	-7.329	6.332	10.922	1.00	15.00	A
ATOM	1082	CA	PHE	229	-8.566	8.106	10.772	1.00	11.18	A
ATOM	1083	CB	PHE	229	-9.578	7.687	9.686	1.00	8.01	A
ATOM	1084	CG	PHE	229	-9.063	7.912	8.233	1.00	8.40	A
ATOM	1085	CD1	PHE	229	-9.140	9.196	7.649	1.00	10.03	A
ATOM	1086	CD2	PHE	229	-8.433	6.883	7.517	1.00	6.57	A
ATOM	1087	CE1	PHE	229	-8.512	9.443	6.395	1.00	5.18	A
ATOM	1088	CE2	PHE	229	-7.771	7.128	6.282	1.00	4.26	A
ATOM	1089	CZ	PHE	229	-7.813	8.424	5.731	1.00	5.71	A
ATOM	1090	C	PHE	229	-9.202	8.014	12.197	1.00	14.39	A
ATOM	1091	O	PHE	229	-9.116	7.000	12.870	1.00	13.92	A
ATOM	1092	N	GLU	230	-9.863	9.064	12.672	1.00	17.93	A
ATOM	1093	H	GLU	230	-9.912	9.892	12.113	1.00	15.00	A
ATOM	1094	CA	GLU	230	-10.856	8.944	13.770	1.00	18.08	A
ATOM	1095	CB	GLU	230	-11.218	10.303	14.393	1.00	16.17	A
ATOM	1096	CG	GLU	230	-11.068	10.090	15.889	1.00	27.69	A
ATOM	1097	CD	GLU	230	-12.314	10.091	16.805	1.00	33.06	A
ATOM	1098	OE1	GLU	230	-13.355	10.707	16.552	1.00	38.26	A
ATOM	1099	OE2	GLU	230	-12.218	9.477	17.863	1.00	38.14	A
ATOM	1100	C	GLU	230	-12.225	8.268	13.453	1.00	18.70	A
ATOM	1101	O	GLU	230	-12.967	8.519	12.492	1.00	21.58	A
ATOM	1102	N	LEU	231	-12.542	7.334	14.361	1.00	13.79	A
ATOM	1103	H	LEU	231	-11.840	7.125	15.015	1.00	15.00	A
ATOM	1104	CA	LEU	231	-13.885	6.836	14.330	1.00	13.52	A
ATOM	1105	CB	LEU	231	-13.954	5.378	14.002	1.00	13.90	A
ATOM	1106	CG	LEU	231	-13.199	5.064	12.725	1.00	15.44	A
ATOM	1107	CD1	LEU	231	-13.781	5.712	11.436	1.00	10.24	A
ATOM	1108	CD2	LEU	231	-12.970	3.569	12.769	1.00	11.74	A
ATOM	1109	C	LEU	231	-14.638	7.074	15.591	1.00	14.88	A
ATOM	1110	O	LEU	231	-14.145	6.912	16.692	1.00	12.46	A
ATOM	1111	N	GLN	232	-15.891	7.411	15.350	1.00	19.40	A
ATOM	1112	H	GLN	232	-16.107	7.560	14.394	1.00	15.00	A
ATOM	1113	CA	GLN	232	-16.920	7.509	16.389	1.00	21.07	A
ATOM	1114	CB	GLN	232	-18.132	8.234	15.804	1.00	23.55	A
ATOM	1115	CG	GLN	232	-17.792	9.709	15.687	1.00	28.60	A
ATOM	1116	CD	GLN	232	-17.625	10.200	17.102	1.00	33.66	A
ATOM	1117	OE1	GLN	232	-18.623	10.472	17.742	1.00	38.08	A
ATOM	1118	NE2	GLN	232	-16.380	10.254	17.596	1.00	33.41	A
ATOM	1119	HE21	GLN	232	-15.596	10.186	16.972	1.00	15.00	A
ATOM	1120	HE22	GLN	232	-16.387	10.470	18.576	1.00	15.00	A
ATOM	1121	C	GLN	232	-17.402	6.148	16.851	1.00	21.86	A
ATOM	1122	O	GLN	232	-17.368	5.218	16.052	1.00	21.58	A
ATOM	1123	N	PRO	233	-17.906	6.013	18.115	1.00	22.31	A
ATOM	1124	CD	PRO	233	-17.962	7.033	19.168	1.00	21.41	A
ATOM	1125	CA	PRO	233	-18.570	4.747	18.442	1.00	21.21	A
ATOM	1126	CB	PRO	233	-19.013	4.987	19.866	1.00	23.88	A
ATOM	1127	CG	PRO	233	-18.661	6.404	20.339	1.00	20.95	A
ATOM	1128	C	PRO	233	-19.667	4.417	17.434	1.00	23.66	A
ATOM	1129	O	PRO	233	-20.275	5.319	16.875	1.00	26.89	A
ATOM	1130	N	GLY	234	-19.731	3.140	17.059	1.00	22.77	A
ATOM	1131	H	GLY	234	-19.082	2.466	17.417	1.00	15.00	A
ATOM	1132	CA	GLY	234	-20.766	2.767	16.072	1.00	19.45	A
ATOM	1133	C	GLY	234	-20.545	3.241	14.625	1.00	19.67	A
ATOM	1134	O	GLY	234	-21.299	2.980	13.715	1.00	23.81	A
ATOM	1135	N	ALA	235	-19.405	3.926	14.368	1.00	18.89	A
ATOM	1136	H	ALA	235	-19.096	4.485	15.135	1.00	15.00	A
ATOM	1137	CA	ALA	235	-18.431	3.515	13.296	1.00	22.17	A
ATOM	1138	CB	ALA	235	-18.193	2.042	13.039	1.00	6.68	A
ATOM	1139	C	ALA	235	-18.540	4.160	11.993	1.00	21.96	A

ATOM	1140	O	ALA	235	-18.486	5.385	12.100	1.00	26.42	A
ATOM	1141	N	SER	236	-18.699	3.498	10.787	1.00	20.94	A
ATOM	1142	H	SER	236	-18.824	4.326	10.254	1.00	15.00	A
ATOM	1143	CA	SER	236	-18.630	2.227	9.961	1.00	17.60	A
ATOM	1144	CB	SER	236	-19.905	1.876	9.160	1.00	14.98	A
ATOM	1145	OG	SER	236	-20.662	0.908	9.833	1.00	21.35	A
ATOM	1146	HG	SER	236	-21.599	0.910	9.647	1.00	15.00	A
ATOM	1147	C	SER	236	-17.794	2.538	8.714	1.00	13.65	A
ATOM	1148	O	SER	236	-17.939	3.614	8.131	1.00	16.29	A
ATOM	1149	N	VAL	237	-16.986	1.567	8.286	1.00	14.95	A
ATOM	1150	H	VAL	237	-16.764	0.823	8.949	1.00	15.00	A
ATOM	1151	CA	VAL	237	-16.201	1.802	7.077	1.00	11.42	A
ATOM	1152	CB	VAL	237	-14.681	2.004	7.284	1.00	12.49	A
ATOM	1153	CG1	VAL	237	-14.113	0.726	7.939	1.00	13.10	A
ATOM	1154	CG2	VAL	237	-14.254	3.396	7.846	1.00	10.27	A
ATOM	1155	C	VAL	237	-16.468	0.746	6.035	1.00	8.76	A
ATOM	1156	O	VAL	237	-16.827	-0.363	6.341	1.00	12.84	A
ATOM	1157	N	PHE	238	-16.354	1.158	4.773	1.00	12.45	A
ATOM	1158	H	PHE	238	-16.139	2.128	4.652	1.00	15.00	A
ATOM	1159	CA	PHE	238	-16.521	0.213	3.653	1.00	11.21	A
ATOM	1160	CB	PHE	238	-18.013	0.137	3.322	1.00	13.00	A
ATOM	1161	CG	PHE	238	-18.634	1.468	2.899	1.00	12.17	A
ATOM	1162	CD1	PHE	238	-18.763	1.812	1.518	1.00	12.94	A
ATOM	1163	CD2	PHE	238	-19.135	2.332	3.887	1.00	10.55	A
ATOM	1164	CE1	PHE	238	-19.407	3.010	1.092	1.00	14.01	A
ATOM	1165	CE2	PHE	238	-19.786	3.504	3.470	1.00	12.74	A
ATOM	1166	CZ	PHE	238	-19.917	3.836	2.100	1.00	13.17	A
ATOM	1167	C	PHE	238	-15.725	0.582	2.379	1.00	11.20	A
ATOM	1168	O	PHE	238	-15.137	1.638	2.267	1.00	8.73	A
ATOM	1169	N	VAL	239	-15.726	-0.300	1.383	1.00	14.34	A
ATOM	1170	H	VAL	239	-16.187	-1.170	1.523	1.00	15.00	A
ATOM	1171	CA	VAL	239	-14.982	0.027	0.154	1.00	14.65	A
ATOM	1172	CB	VAL	239	-13.900	-1.043	-0.162	1.00	14.09	A
ATOM	1173	CG1	VAL	239	-13.004	-1.318	1.038	1.00	14.55	A
ATOM	1174	CG2	VAL	239	-13.064	-0.594	-1.361	1.00	14.74	A
ATOM	1175	C	VAL	239	-15.930	0.081	-1.043	1.00	18.32	A
ATOM	1176	O	VAL	239	-16.558	-0.903	-1.369	1.00	18.99	A
ATOM	1177	N	ASN	240	-16.000	1.207	-1.707	1.00	19.26	A
ATOM	1178	H	ASN	240	-15.420	1.947	-1.383	1.00	15.00	A
ATOM	1179	CA	ASN	240	-16.613	1.355	-3.031	1.00	21.66	A
ATOM	1180	CB	ASN	240	-16.850	2.856	-3.095	1.00	24.58	A
ATOM	1181	CG	ASN	240	-18.167	3.077	-3.708	1.00	29.09	A
ATOM	1182	OD1	ASN	240	-18.948	2.123	-3.740	1.00	35.44	A
ATOM	1183	ND2	ASN	240	-18.293	4.331	-4.166	1.00	34.71	A
ATOM	1184	HD21	ASN	240	-19.149	4.489	-4.657	1.00	15.00	A
ATOM	1185	C	ASN	240	-15.669	0.950	-4.184	1.00	20.96	A
ATOM	1186	O	ASN	240	-14.473	1.128	-4.058	1.00	20.99	A
ATOM	1187	N	VAL	241	-16.189	0.383	-5.275	1.00	21.52	A
ATOM	1188	H	VAL	241	-17.182	0.230	-5.295	1.00	15.00	A
ATOM	1189	CA	VAL	241	-15.387	0.439	-6.516	1.00	20.56	A
ATOM	1190	CB	VAL	241	-14.581	-0.850	-6.849	1.00	18.02	A
ATOM	1191	CG1	VAL	241	-15.501	-2.058	-7.063	1.00	15.06	A
ATOM	1192	CG2	VAL	241	-13.597	-1.259	-5.764	1.00	20.05	A
ATOM	1193	C	VAL	241	-16.253	0.758	-7.741	1.00	18.88	A
ATOM	1194	O	VAL	241	-17.441	0.500	-7.819	1.00	18.63	A
ATOM	1195	N	THR	242	-15.541	1.162	-8.762	1.00	21.24	A
ATOM	1196	H	THR	242	-14.704	1.653	-8.486	1.00	15.00	A
ATOM	1197	CA	THR	242	-16.246	1.476	-10.031	1.00	20.63	A
ATOM	1198	CB	THR	242	-15.342	2.269	-10.981	1.00	15.80	A
ATOM	1199	OG1	THR	242	-14.035	1.663	-10.953	1.00	17.72	A

ATOM	1200	HG1	THR	242	-13.721	1.969	-11.812	1.00	15.00	A
ATOM	1201	CG2	THR	242	-15.238	3.732	-10.650	1.00	15.04	A
ATOM	1202	C	THR	242	-16.755	0.240	-10.783	1.00	18.92	A
ATOM	1203	O	THR	242	-17.846	0.198	-11.297	1.00	21.26	A
ATOM	1204	N	ASP	243	-15.923	-0.806	-10.718	1.00	20.98	A
ATOM	1205	H	ASP	243	-15.087	-0.580	-10.221	1.00	15.00	A
ATOM	1206	CA	ASP	243	-16.092	-1.977	-11.628	1.00	21.28	A
ATOM	1207	CB	ASP	243	-14.905	-2.126	-12.594	1.00	22.05	A
ATOM	1208	CG	ASP	243	-14.932	-0.954	-13.492	1.00	28.23	A
ATOM	1209	OD1	ASP	243	-14.314	0.051	-13.115	1.00	28.43	A
ATOM	1210	OD2	ASP	243	-15.588	-1.033	-14.535	1.00	33.00	A
ATOM	1211	C	ASP	243	-16.123	-3.308	-10.923	1.00	20.38	A
ATOM	1212	O	ASP	243	-15.148	-4.072	-10.967	1.00	20.43	A
ATOM	1213	N	PRO	244	-17.204	-3.553	-10.154	1.00	19.92	A
ATOM	1214	CD	PRO	244	-18.481	-2.871	-10.071	1.00	16.83	A
ATOM	1215	CA	PRO	244	-17.120	-4.706	-9.269	1.00	19.13	A
ATOM	1216	CB	PRO	244	-18.293	-4.535	-8.275	1.00	15.33	A
ATOM	1217	CG	PRO	244	-18.890	-3.174	-8.634	1.00	15.21	A
ATOM	1218	C	PRO	244	-16.975	-6.034	-9.974	1.00	19.29	A
ATOM	1219	O	PRO	244	-16.194	-6.859	-9.548	1.00	23.48	A
ATOM	1220	N	SER	245	-17.581	-6.163	-11.150	1.00	22.60	A
ATOM	1221	H	SER	245	-18.220	-5.459	-11.473	1.00	15.00	A
ATOM	1222	CA	SER	245	-17.414	-7.429	-11.942	1.00	25.50	A
ATOM	1223	CB	SER	245	-18.256	-7.369	-13.234	1.00	21.36	A
ATOM	1224	OG	SER	245	-19.667	-7.567	-12.981	1.00	38.26	A
ATOM	1225	HG	SER	245	-19.848	-7.390	-12.038	1.00	15.00	A
ATOM	1226	C	SER	245	-15.955	-7.776	-12.328	1.00	24.14	A
ATOM	1227	O	SER	245	-15.477	-8.859	-12.623	1.00	24.84	A
ATOM	1228	N	GLN	246	-15.177	-6.689	-12.385	1.00	28.52	A
ATOM	1229	H	GLN	246	-15.638	-5.804	-12.265	1.00	15.00	A
ATOM	1230	CA	GLN	246	-13.743	-6.923	-12.590	1.00	26.45	A
ATOM	1231	CB	GLN	246	-13.144	-5.645	-13.233	1.00	29.90	A
ATOM	1232	CG	GLN	246	-13.403	-5.435	-14.758	1.00	26.84	A
ATOM	1233	CD	GLN	246	-14.862	-5.341	-15.129	1.00	21.60	A
ATOM	1234	OE1	GLN	246	-15.538	-4.503	-14.616	1.00	24.20	A
ATOM	1235	NE2	GLN	246	-15.334	-6.234	-15.975	1.00	26.15	A
ATOM	1236	HE21	GLN	246	-14.763	-6.924	-16.423	1.00	15.00	A
ATOM	1237	HE22	GLN	246	-16.320	-6.119	-16.084	1.00	15.00	A
ATOM	1238	C	GLN	246	-12.936	-7.372	-11.363	1.00	27.14	A
ATOM	1239	O	GLN	246	-11.721	-7.570	-11.454	1.00	25.73	A
ATOM	1240	N	VAL	247	-13.615	-7.395	-10.196	1.00	23.70	A
ATOM	1241	H	VAL	247	-14.600	-7.594	-10.146	1.00	15.00	A
ATOM	1242	CA	VAL	247	-12.728	-7.569	-9.097	1.00	21.91	A
ATOM	1243	CB	VAL	247	-13.156	-6.814	-7.859	1.00	21.59	A
ATOM	1244	CG1	VAL	247	-14.027	-7.616	-6.962	1.00	24.52	A
ATOM	1245	CG2	VAL	247	-13.680	-5.409	-8.167	1.00	21.61	A
ATOM	1246	C	VAL	247	-12.258	-8.998	-8.910	1.00	21.55	A
ATOM	1247	O	VAL	247	-12.946	-9.912	-9.251	1.00	19.53	A
ATOM	1248	N	SER	248	-11.000	-9.152	-8.444	1.00	21.31	A
ATOM	1249	H	SER	248	-10.558	-8.342	-8.070	1.00	15.00	A
ATOM	1250	CA	SER	248	-10.414	-10.499	-8.327	1.00	21.97	A
ATOM	1251	CB	SER	248	-8.939	-10.571	-8.828	1.00	23.61	A
ATOM	1252	OG	SER	248	-8.860	-9.952	-10.128	1.00	20.21	A
ATOM	1253	HG	SER	248	-9.752	-10.027	-10.496	1.00	15.00	A
ATOM	1254	C	SER	248	-10.538	-11.076	-6.946	1.00	19.28	A
ATOM	1255	O	SER	248	-10.048	-10.409	-6.052	1.00	20.64	A
ATOM	1256	N	HIS	249	-11.269	-12.204	-6.814	1.00	18.72	A
ATOM	1257	H	HIS	249	-11.284	-12.753	-7.674	1.00	15.00	A
ATOM	1258	CA	HIS	249	-11.640	-12.673	-5.478	1.00	17.22	A
ATOM	1259	CB	HIS	249	-13.080	-13.152	-5.484	1.00	13.10	A

ATOM	1260	CG	HIS	249	-13.919	-11.905	-5.550	1.00	10.13	A
ATOM	1261	ND1	HIS	249	-14.137	-11.129	-4.486	1.00	13.47	A
ATOM	1262	HD1	HIS	249	-13.720	-11.294	-3.611	1.00	15.00	A
ATOM	1263	CD2	HIS	249	-14.662	-11.414	-6.610	1.00	10.62	A
ATOM	1264	NE2	HIS	249	-15.317	-10.347	-6.134	1.00	15.51	A
ATOM	1265	CE1	HIS	249	-15.018	-10.142	-4.821	1.00	12.36	A
ATOM	1266	C	HIS	249	-10.701	-13.683	-4.858	1.00	23.58	A
ATOM	1267	O	HIS	249	-11.103	-14.729	-4.359	1.00	21.98	A
ATOM	1268	N	GLY	250	-9.398	-13.258	-4.878	1.00	29.10	A
ATOM	1269	H	GLY	250	-9.252	-12.351	-5.253	1.00	15.00	A
ATOM	1270	CA	GLY	250	-8.410	-14.041	-4.115	1.00	24.27	A
ATOM	1271	C	GLY	250	-8.336	-15.372	-4.743	1.00	25.93	A
ATOM	1272	O	GLY	250	-8.940	-15.520	-5.795	1.00	29.26	A
ATOM	1273	N	THR	251	-7.594	-16.302	-4.127	1.00	22.38	A
ATOM	1274	H	THR	251	-7.485	-17.038	-4.804	1.00	15.00	A
ATOM	1275	CA	THR	251	-7.111	-16.139	-2.725	1.00	21.12	A
ATOM	1276	CB	THR	251	-6.988	-17.525	-1.933	1.00	24.76	A
ATOM	1277	OG1	THR	251	-5.877	-17.641	-0.981	1.00	22.90	A
ATOM	1278	HG1	THR	251	-6.063	-18.366	-0.381	1.00	15.00	A
ATOM	1279	CG2	THR	251	-6.968	-18.722	-2.890	1.00	22.77	A
ATOM	1280	C	THR	251	-5.952	-15.158	-2.473	1.00	17.96	A
ATOM	1281	O	THR	251	-4.969	-15.043	-3.213	1.00	12.30	A
ATOM	1282	N	GLY	252	-6.241	-14.367	-1.419	1.00	16.85	A
ATOM	1283	H	GLY	252	-7.093	-14.432	-0.862	1.00	15.00	A
ATOM	1284	CA	GLY	252	-5.277	-13.375	-0.928	1.00	13.16	A
ATOM	1285	C	GLY	252	-5.357	-12.058	-1.670	1.00	15.51	A
ATOM	1286	O	GLY	252	-4.580	-11.168	-1.439	1.00	15.18	A
ATOM	1287	N	PHE	253	-6.189	-12.063	-2.744	1.00	16.66	A
ATOM	1288	H	PHE	253	-6.868	-12.805	-2.761	1.00	15.00	A
ATOM	1289	CA	PHE	253	-6.110	-10.892	-3.651	1.00	15.77	A
ATOM	1290	CB	PHE	253	-6.649	-11.216	-5.100	1.00	17.11	A
ATOM	1291	CG	PHE	253	-5.595	-11.840	-5.994	1.00	11.82	A
ATOM	1292	CD1	PHE	253	-4.385	-11.175	-6.231	1.00	13.69	A
ATOM	1293	CD2	PHE	253	-5.845	-13.089	-6.558	1.00	18.59	A
ATOM	1294	CE1	PHE	253	-3.364	-11.771	-6.993	1.00	14.39	A
ATOM	1295	CE2	PHE	253	-4.840	-13.680	-7.363	1.00	21.37	A
ATOM	1296	CZ	PHE	253	-3.612	-13.014	-7.562	1.00	15.72	A
ATOM	1297	C	PHE	253	-6.740	-9.599	-3.147	1.00	13.88	A
ATOM	1298	O	PHE	253	-6.347	-8.477	-3.453	1.00	14.27	A
ATOM	1299	N	THR	254	-7.865	-9.837	-2.502	1.00	14.00	A
ATOM	1300	H	THR	254	-8.079	-10.748	-2.124	1.00	15.00	A
ATOM	1301	CA	THR	254	-8.741	-8.681	-2.185	1.00	14.09	A
ATOM	1302	CB	THR	254	-9.908	-8.469	-3.201	1.00	11.66	A
ATOM	1303	OG1	THR	254	-9.414	-8.325	-4.536	1.00	13.08	A
ATOM	1304	HG1	THR	254	-9.826	-9.054	-4.992	1.00	15.00	A
ATOM	1305	CG2	THR	254	-10.882	-7.321	-2.885	1.00	13.78	A
ATOM	1306	C	THR	254	-9.270	-8.779	-0.738	1.00	12.36	A
ATOM	1307	O	THR	254	-9.906	-9.695	-0.240	1.00	14.54	A
ATOM	1308	N	SER	255	-9.007	-7.683	-0.027	1.00	13.42	A
ATOM	1309	H	SER	255	-8.425	-7.021	-0.490	1.00	15.00	A
ATOM	1310	CA	SER	255	-9.032	-7.725	1.431	1.00	7.59	A
ATOM	1311	CB	SER	255	-7.793	-8.466	1.976	1.00	6.39	A
ATOM	1312	OG	SER	255	-6.704	-7.560	2.041	1.00	9.69	A
ATOM	1313	HG	SER	255	-5.920	-8.031	1.741	1.00	15.00	A
ATOM	1314	C	SER	255	-9.248	-6.341	2.085	1.00	10.05	A
ATOM	1315	O	SER	255	-9.191	-5.254	1.492	1.00	15.21	A
ATOM	1316	N	PHE	256	-9.653	-6.385	3.369	1.00	8.54	A
ATOM	1317	H	PHE	256	-9.700	-7.323	3.733	1.00	15.00	A
ATOM	1318	CA	PHE	256	-10.114	-5.168	4.035	1.00	7.94	A
ATOM	1319	CB	PHE	256	-11.605	-5.009	3.679	1.00	11.65	A

ATOM	1320	CG	PHE	256	-12.376	-3.824	4.235	1.00	8.72	A
ATOM	1321	CD1	PHE	256	-11.766	-2.570	4.533	1.00	11.20	A
ATOM	1322	CD2	PHE	256	-13.756	-3.976	4.327	1.00	6.12	A
ATOM	1323	CE1	PHE	256	-12.503	-1.490	5.034	1.00	11.49	A
ATOM	1324	CE2	PHE	256	-14.514	-2.849	4.734	1.00	6.86	A
ATOM	1325	CZ	PHE	256	-13.862	-1.657	5.211	1.00	9.27	A
ATOM	1326	C	PHE	256	-9.933	-5.268	5.560	1.00	11.92	A
ATOM	1327	O	PHE	256	-10.195	-6.290	6.177	1.00	9.43	A
ATOM	1328	N	GLY	257	-9.420	-4.207	6.169	1.00	10.57	A
ATOM	1329	H	GLY	257	-9.217	-3.365	5.653	1.00	15.00	A
ATOM	1330	CA	GLY	257	-9.368	-4.406	7.612	1.00	11.26	A
ATOM	1331	C	GLY	257	-8.965	-3.122	8.287	1.00	11.14	A
ATOM	1332	O	GLY	257	-8.916	-2.068	7.679	1.00	10.81	A
ATOM	1333	N	LEU	258	-8.688	-3.277	9.565	1.00	12.61	A
ATOM	1334	H	LEU	258	-8.776	-4.204	9.943	1.00	15.00	A
ATOM	1335	CA	LEU	258	-8.434	-2.098	10.426	1.00	14.72	A
ATOM	1336	CB	LEU	258	-9.751	-1.212	10.704	1.00	14.67	A
ATOM	1337	CG	LEU	258	-10.991	-1.863	11.379	1.00	18.02	A
ATOM	1338	CD1	LEU	258	-12.317	-1.125	11.094	1.00	15.05	A
ATOM	1339	CD2	LEU	258	-10.743	-2.047	12.905	1.00	15.42	A
ATOM	1340	C	LEU	258	-7.737	-2.525	11.709	1.00	11.84	A
ATOM	1341	O	LEU	258	-7.851	-3.690	12.096	1.00	7.91	A
ATOM	1342	N	LEU	259	-7.058	-1.537	12.343	1.00	11.64	A
ATOM	1343	H	LEU	259	-6.883	-0.685	11.844	1.00	15.00	A
ATOM	1344	CA	LEU	259	-6.581	-1.780	13.714	1.00	9.53	A
ATOM	1345	CB	LEU	259	-5.155	-2.417	13.831	1.00	7.40	A
ATOM	1346	CG	LEU	259	-4.194	-1.621	12.931	1.00	11.40	A
ATOM	1347	CD1	LEU	259	-3.355	-2.412	11.926	1.00	7.83	A
ATOM	1348	CD2	LEU	259	-3.379	-0.670	13.808	1.00	13.30	A
ATOM	1349	C	LEU	259	-6.652	-0.497	14.531	1.00	10.40	A
ATOM	1350	O	LEU	259	-6.202	0.556	14.082	1.00	9.73	A
ATOM	1351	N	LYS	260	-7.193	-0.629	15.762	1.00	12.00	A
ATOM	1352	H	LYS	260	-7.395	-1.553	16.115	1.00	15.00	A
ATOM	1353	CA	LYS	260	-7.069	0.521	16.693	1.00	13.51	A
ATOM	1354	CB	LYS	260	-8.014	0.312	17.885	1.00	13.49	A
ATOM	1355	CG	LYS	260	-8.378	1.656	18.521	1.00	17.16	A
ATOM	1356	CD	LYS	260	-9.435	1.456	19.596	1.00	12.01	A
ATOM	1357	CE	LYS	260	-10.151	2.681	20.121	1.00	11.41	A
ATOM	1358	NZ	LYS	260	-9.175	3.595	20.697	1.00	13.33	A
ATOM	1359	HZ1	LYS	260	-8.534	3.932	19.954	1.00	15.00	A
ATOM	1360	HZ2	LYS	260	-9.693	4.404	21.095	1.00	15.00	A
ATOM	1361	HZ3	LYS	260	-8.638	3.136	21.458	1.00	15.00	A
ATOM	1362	C	LYS	260	-5.648	0.921	17.125	1.00	16.54	A
ATOM	1363	O	LYS	260	-4.828	0.112	17.481	1.00	15.61	A
ATOM	1364	N	LEU	261	-5.353	2.199	17.015	1.00	14.78	A
ATOM	1365	H	LEU	261	-6.089	2.838	16.856	1.00	15.00	A
ATOM	1366	CB	LEU	261	-3.705	4.005	17.185	1.00	19.53	A
ATOM	1367	CG	LEU	261	-3.177	4.309	15.787	1.00	16.82	A
ATOM	1368	CD1	LEU	261	-3.010	5.779	15.767	1.00	12.45	A
ATOM	1369	CD2	LEU	261	-4.010	3.906	14.577	1.00	18.20	A
ATOM	1370	C	LEU	261	-4.243	2.667	19.225	1.00	20.80	A
ATOM	1371	OCT1	LEU	261	-5.363	2.741	19.746	1.00	22.59	A
ATOM	1372	OCT2	LEU	261	-3.221	2.696	19.913	1.00	26.97	A
ATOM	1373	CA	LEU	261	-4.122	2.604	17.684	1.00	18.13	A
ATOM	1374	O	HOH	501	-20.040	9.837	7.596	1.00	16.33	W
ATOM	1375	H1	HOH	501	-19.411	10.547	7.803	1.00	10.00	W
ATOM	1376	H2	HOH	501	-19.615	9.317	6.900	1.00	10.00	W
ATOM	1377	O	HOH	502	-9.727	11.545	10.743	1.00	10.94	W
ATOM	1378	H1	HOH	502	-10.039	11.934	9.919	1.00	15.00	W
ATOM	1379	H2	HOH	502	-10.233	12.125	11.315	1.00	15.00	W

ATOM	1380	O	HOH	503	-8.158	13.188	13.681	1.00	30.64	W
ATOM	1381	H1	HOH	503	-8.715	12.529	13.277	1.00	15.00	W
ATOM	1382	H2	HOH	503	-8.700	13.944	13.574	1.00	15.00	W
ATOM	1383	O	HOH	504	-16.772	8.440	12.789	1.00	12.00	W
ATOM	1384	H1	HOH	504	-17.194	9.259	12.886	1.00	10.00	W
ATOM	1385	H2	HOH	504	-15.921	8.763	12.582	1.00	10.00	W
ATOM	1386	O	HOH	505	-25.173	7.297	7.925	1.00	47.03	W
ATOM	1387	H1	HOH	505	-24.690	8.064	8.239	1.00	10.00	W
ATOM	1388	H2	HOH	505	-25.990	7.684	7.583	1.00	10.00	W
ATOM	1389	O	HOH	506	-23.612	14.948	13.859	1.00	36.14	W
ATOM	1390	H1	HOH	506	-24.160	15.702	13.605	1.00	10.00	W
ATOM	1391	H2	HOH	506	-23.282	15.191	14.748	1.00	10.00	W
ATOM	1392	O	HOH	507	-17.329	-8.460	-7.186	1.00	34.02	W
ATOM	1393	O	HOH	508	-18.687	-7.253	-3.843	1.00	63.14	W
ATOM	1394	O	HOH	509	-7.157	11.327	3.239	1.00	22.26	W
ATOM	1395	O	HOH	510	-19.322	7.486	-2.227	1.00	37.69	W
ATOM	1396	O	HOH	511	-14.645	-7.711	-1.931	1.00	26.48	W
ATOM	1397	O	HOH	512	-18.377	-9.754	12.556	1.00	24.86	W
ATOM	1398	O	HOH	513	0.030	0.048	-13.455	1.00	26.05	W
ATOM	1399	O	HOH	514	-8.938	5.945	22.862	1.00	34.39	W
ATOM	1400	O	HOH	515	-29.446	-4.922	-7.247	1.00	41.61	W
ATOM	1401	O	HOH	516	-12.982	10.220	10.038	1.00	47.16	W
ATOM	1402	O	HOH	517	-21.797	-9.377	7.242	1.00	60.65	W
ATOM	1403	O	HOH	518	-7.867	8.165	19.484	1.00	40.46	W
ATOM	1404	O	HOH	520	-15.588	-14.701	14.628	1.00	63.80	W
ATOM	1405	O	HOH	521	-21.844	7.778	20.415	1.00	35.72	W
ATOM	1406	O	HOH	522	-6.555	-3.308	-15.790	1.00	33.63	W
ATOM	1407	O	HOH	523	-9.046	-13.476	-8.051	1.00	44.08	W
ATOM	1408	O	HOH	524	-17.413	-9.311	17.071	1.00	34.06	W
ATOM	1409	O	HOH	525	-23.838	4.781	19.884	1.00	37.99	W
ATOM	1410	O	HOH	526	-26.323	15.525	10.379	1.00	72.49	W
ATOM	1411	O	HOH	527	-3.167	-13.749	-10.820	1.00	43.99	W
ATOM	1412	O	HOH	528	-0.470	2.513	17.943	1.00	63.68	W
ATOM	1413	O	HOH	529	-5.580	-12.778	-14.864	1.00	47.52	W
ATOM	1414	O	HOH	530	-2.641	7.004	2.495	1.00	18.07	W
ATOM	1415	O	HOH	531	-6.472	12.847	0.156	1.00	24.96	W
ATOM	1416	O	HOH	532	-10.363	-16.426	-0.360	1.00	63.56	W
ATOM	1417	O	HOH	533	-1.378	-17.183	-13.053	1.00	67.67	W
ATOM	1418	O	HOH	534	-4.774	9.073	-0.651	1.00	23.36	W
ATOM	1419	O	HOH	535	-18.917	-13.857	6.913	1.00	32.28	W
ATOM	1420	O	HOH	536	-23.062	3.270	0.454	1.00	52.03	W
ATOM	1421	O	HOH	537	-25.906	9.022	16.986	1.00	44.75	W
ATOM	1422	O	HOH	538	-21.729	16.972	17.027	1.00	53.12	W
ATOM	1423	O	HOH	539	-9.084	11.806	17.034	1.00	70.90	W
ATOM	1424	O	HOH	540	-10.938	-13.296	15.207	1.00	35.65	W
ATOM	1425	O	HOH	541	-6.068	13.255	17.989	1.00	67.36	W
ATOM	1426	O	HOH	542	-20.593	-11.039	-9.003	1.00	96.30	W
ATOM	1427	O	HOH	543	-15.926	13.397	1.269	1.00	35.72	W
ATOM	1428	O	HOH	544	-24.591	-7.285	-2.353	1.00	43.42	W
ATOM	1429	O	HOH	545	-25.859	-2.666	-15.747	1.00	53.56	W
ATOM	1430	O	HOH	546	-23.074	-1.533	11.026	1.00	56.44	W
ATOM	1431	O	HOH	548	-8.941	-12.649	-12.394	1.00	64.34	W
ATOM	1432	O	HOH	549	-14.150	6.038	-12.250	1.00	41.38	W
ATOM	1433	O	HOH	550	-14.274	-0.613	18.441	1.00	56.17	W
ATOM	1434	O	HOH	551	-12.241	-19.609	8.637	1.00	80.90	W
ATOM	1435	O	HOH	552	-10.316	15.578	10.166	1.00	39.58	W
ATOM	1436	O	HOH	553	-15.367	10.941	14.659	1.00	40.40	W
ATOM	1437	O	HOH	554	-2.322	1.830	-5.294	1.00	33.65	W
ATOM	1438	O	HOH	555	-22.393	-14.875	-4.217	1.00	52.40	W
ATOM	1439	O	HOH	556	-22.120	14.279	7.189	1.00	38.55	W

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ATOM   1440  O   HOH   557     -28.833   6.135   9.560  1.00 37.40      W
ATOM   1441  O   HOH   558      -5.554 -16.509  13.192  1.00 88.88      W
ATOM   1442  O   HOH   559     -22.996  12.522   1.162  1.00 63.77      W
ATOM   1443  O   HOH   560     -13.764   2.268 -14.743  1.00 27.47      W
ATOM   1444  O   HOH   561     -15.556   7.750  -5.628  1.00 75.88      W
ATOM   1445  O   HOH   562      -1.970 -15.363 -17.719  1.00 76.30      W
ATOM   1446  O   HOH   563     -18.939  -0.335 -13.842  1.00 48.39      W
ATOM   1447  O   HOH   564     -12.619  14.760  -6.974  1.00100.59      W
ATOM   1448  O   HOH   565      -9.491  18.046  13.682  1.00 87.45      W
ATOM   1449  O   HOH   566     -11.655 -11.140  22.481  1.00 28.88      W
ATOM   1450  O   HOH   567     -24.072  -3.264  -0.332  1.00 35.13      W
ATOM   1451  O   HOH   568     -27.455   0.119  -7.117  1.00 71.07      W
ATOM   1452  O   HOH   569     -14.604   3.516  -6.119  1.00 59.45      W
ATOM   1453  O   HOH   570      -2.635  -9.566 -16.973  1.00 59.09      W
ATOM   1454  O   HOH   571     -18.841   4.066  -7.543  1.00 34.10      W
ATOM   1455  O   HOH   572     -24.996   1.301  17.953  1.00 70.45      W
ATOM   1456  O   HOH   573     -14.666  16.471   8.995  1.00 62.77      W
ATOM   1457  O   HOH   574     -14.786   1.426  10.949  1.00 82.68      W
ATOM   1458  O   HOH   575     -16.584 -14.717  -4.352  1.00 29.09      W
ATOM   1459  O   HOH   576     -16.273  -4.590   6.109  1.00104.64      W
ATOM   1460  O   HOH   577     -25.471  -0.127  -2.510  1.00 62.74      W
ATOM   1461  O   HOH   578      -7.334 -17.173  19.514  1.00 89.62      W
ATOM   1462  O   HOH   579     -21.060  14.259  19.996  1.00 69.59      W
ATOM   1463  O   HOH   580     -19.286   4.057 -12.816  1.00 60.37      W
ATOM   1464  O   HOH   581     -22.445 -15.840   0.317  1.00 58.24      W
ATOM   1465  O   HOH   582     -22.434 -10.539  12.489  1.00 70.25      W
ATOM   1466  O   HOH   583     -21.327   3.668  -2.500  1.00 39.32      W
ATOM   1467  O   HOH   584     -25.325   5.247  16.919  1.00 41.31      W
ATOM   1468  O   HOH   585     -24.945 -10.718  -2.375  1.00 38.85      W
ATOM   1469  O   HOH   586     -24.342 -13.003   1.927  1.00 70.58      W
ATOM   1470  O   HOH   587     -18.020  11.871  11.358  1.00 64.47      W
ATOM   1471  O   HOH   588     -27.135   6.965  13.151  1.00 53.96      W
ATOM   1472  O   HOH   589     -14.982 -16.230  -2.494  1.00 30.24      W
ATOM   1473  O   HOH   590      -5.646  14.418  -2.232  1.00 41.78      W
ATOM   1474  O   HOH   591      -2.745  -0.153 -17.104  1.00 55.19      W
ATOM   1475  O   HOH   592      -3.397  -7.012  22.477  1.00 59.46      W
ATOM   1476  O   HOH   593     -32.916  -4.705  -4.143  1.00 51.88      W
ATOM   1477  O   HOH   594     -10.913 -18.855  -3.503  1.00 42.29      W
ATOM   1478  O   HOH   595     -24.157   1.821  -6.165  1.00 47.43      W
END
```

WHAT IS CLAIMED IS:

1. A method of preparing a crystal of CD40 ligand comprising the steps of:
   a) providing an aqueous solution comprising a fragment of CD40 ligand;
   b) providing a reservoir solution comprising a precipitating agent;
   c) mixing a volume of said aqueous solution with a volume of said reservoir solution thereby forming a mixed volume; and
   d) crystallizing at least a portion of said mixed volume.

2. The method of claim 1 wherein the aqueous solution of CD40 ligand provided in step a) has a concentration of CD40 ligand of about 1 to about 50 mg per ml.

3. The method of claim 2 wherein the aqueous solution has a concentration of CD40 ligand of about 5 mg per ml to about 15 mg per ml.

4. The method of claim 3 wherein the aqueous solution has a concentration of CD40 ligand of about 10 mg per ml.

5. The method of claim 1 wherein the precipitating agent is selected from the group consisting of sodium citrate, ammonium sulfate and polyethylene glycol.

6. The method of claim 1 wherein the concentration of the precipitating agent in the reservoir solution is about 1.0 M to about 1.5 M.

7. The method of claim 6 wherein the concentration of precipitating agent is about 1.2 M.

8. The method of claim 1 wherein the pH of the reservoir

solution is about 4 to about 10.

9. The method of claim 8 wherein the pH is about 7.5.

10. The method of claim 1 wherein step d) is by vapor diffusion crystallization, batch crystallization, liquid bridge crystallization or dialysis crystallization.

11. A crystal formed by a functional fragment of the extracellular domain of CD40 ligand having approximately the following cell constants: a+b=77.17Å, c= 90.46Å, $\alpha = \beta = 90°$, $\gamma = 120°$, and a space group of R3.

12. A crystal according to claim 11 described by the structural coordinates identified in Figure 10.

13. A machine readable data storage medium comprising a data storage material encoded with machine readable data which, when read by an appropriate machine, is capable of displaying a three dimensional representation of a crystal of a molecule or molecular complex comprising a fragment of CD40L having a binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

14. A crystal of CD40 ligand (116-261) according to claim 11, or a homolog thereof, wherein said crystal comprises a binding site, said binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

15. A crystal according to claim 11 or a homolog thereof wherein said the crystal comprises Arg207 in close proximity to at least two hydrophobic residues.

16. A crystal according to claim 14 wherein said crystal comprises at least 30 amino acids selected from the group consisting of: Ile127, Ser128, Glu129, Ala130, Ser131, Thr135, Ser136, Ala41, Lu142, Gly144, Tyr146, Cys178, Asn180, Ser185, Gln186, Ala187, Pro188, Ile190, Ala191, Ser192, Ser197, Pro198, Gly199, Arg200, Phe201, Glu202, Ile204, Ala209, Thr211, Pro217, Cys218, Gly219, Gln220, Glu230, Leu231, Gln232, Asn240, Val241, Thr242, Asp243, Ser245, Val247, Ser248, His249, Gly250, Thr251, Gly252 and Phe253.

17. A crystal according to claim 16, wherein said binding site comprises amino acids Ile127, Ser128, Glu129, Ala130, Ser131, Thr135, Ser136, Ala141, Glu142, Lys143, Gly144, Tyr145, Tyr146, Cys178, Asn180, Ser185, Gln186, Ala187, Pro188, Ile190, Ala191, Ser192, Ser197, Pro198, Gly199, Arg200, Phe201, Glu202, Arg203, Ile204, Arg207, Ala209, Thr211, Pro217, Cys218, Gly219, Gln220, Glu230, Leu231, Gln232, Asn240, Val241, Thr242, Asp243, Ser245, Val247, Ser248, His249, Gly250, Thr251, Gly252 and Phe253.

18. A method for determining at least a portion of a three dimensional structure of a molecular complex, said complex comprising at least a fragment of CD40 ligand, said method comprising the steps of:

a.) determining the structural coordinates of a crystal of the fragment of CD40 ligand;

b.) calculating phases from the structural coordinates;

c) calculating an electron density map from the phases obtained in step b);

d) determining the structure of at least a portion of the complex based upon said electron density map.

19. The method of claim 18 wherein the structural coordinates used in step a) are (1) substantially the same as those described in Table 1 or (2) describe substantially the same crystal as the coordinates in Table 1.

20. A method for evaluating the ability of a chemical entity to associate with CD40 ligand or CD40, a fragment of CD40 or CD40 ligand, or a complex comprising CD40 ligand, CD40, or homologs thereof, said method comprising the steps of:
a) employing computational or experimental means to perform a fitting operation between the chemical entity and said CD40 ligand or CD40, fragment or complex thereof, thereby obtaining data related to said association; and
b) analyzing the data obtained in step a) to determine the characteristics of the association between the chemical entity and said CD40 ligand or CD40, fragment or complex.

21. A chemical entity identified by the method of claim 20, wherein said chemical entity is capable of interfering with the in vivo or in vitro association between CD40 and CD40L.

22. A chemical entity identified by the method of claim 20, wherein said chemical entity is capable of associating with a binding site on CD40L, said binding site comprising amino acids Lys143, Arg203, Arg207 and Tyr145.

23. A chemical entity identified by the method of claim 20 wherein said chemical entity is capable of associating with CD40, and comprises a binding site comprising

amino acids Lys143, Arg203, Arg207 and Tyr145.

24. The chemical entity identified by the method of claim 22 or 23 wherein said CD40L binding site comprises at least 30 amino acids selected from the group consisting of: Ile127, Ser128, Glu129, Ala130, Ser131, Thr135, Ser136, Ala141, Glu142, Gly144, Tyr146, Cys178, Asn180, Ser185, Gln186, Ala187, Pro188, Ile190, Ala191, Ser192, Ser197, Pro198, Gly199, Arg200, Phe201, Glu202, Ile204, Ala209, Thr211, Pro217, Cys218, Gly219, Gln220, Glu230, Leu231, Gln232, Asn240, Val241, Thr242, Asp243, Ser245, Val247, Ser248, His249, Gly250, Thr251, Gly252 and Phe253.

25. The chemical entity of claim 23, wherein said entity is capable of associating with a binding site on CD40L, wherein said binding site comprises amino acids Ile127, Ser128, Glu129, Ala130, Ser131, Thr135, Ser136, Ala141, Glu142, Lys143, Gly144, Tyr145, Tyr146, Cys178, Asn180, Ser185, Gln186, Ala187, Pro188, Ile190, Ala191, Ser192, Ser197, Pro198, Gly199, Arg200, Phe201, Glu202, Arg203, Ile204, Arg207, Ala209, Thr211, Pro217, Cys218, Gly219, Gln220, Glu230, Leu231, Gln232, Asn240, Val241, Thr242, Asp243, Ser245, Val247, Ser248, His249, Gly250, Thr251, Gly252 and Phe253.

26. A heavy atom derivative of a crystallized form of CD40 ligand.

27. A heavy atom derivative of the crystal of claim 11.

28. The use of the structural coordinates of CD40 ligand, or portions thereof, to solve a crystal form of a mutant, homologue or co-complex of CD40 ligand or a fragment thereof by molecular replacement.

29. A method of computationally or experimentally evaluating a chemical entity to obtain information about its association with the binding site of CD40 ligand using a crystal of CD40 ligand or the structural coordinates thereof.

30. The method of claim 29 wherein the crystal has the structural coordinates described in Table 1.

31. The use of the structural coordinates of a crystal, wherein said crystal is substantially the same as the crystal of CD40 ligand described by the coordinates in Table 1.

32. The method of claim 29 wherein said crystal is a crystal according to claim 11.

33. The use of the structural coordinates of a crystal according to claim 31, to identify, characterize or design chemical entities having a desired association with a CD40 ligand, or fragment thereof.

34. The method of claim 33 further comprising the step of optimizing the binding characteristics of the chemical entity identified, characterized, or designed.

35. The method of claim 34 further comprising the step of determining the orientation of ligands in a binding site of CD40 ligand.

36. A chemical entity identified or designed according to claim 33.

37. The use of a CD40 ligand crystal to determine binding interactions between a chemical entity and CD40 ligand.

38. The use according to claim 37 wherein said CD40 ligand crystal is the crystal of claim 11.

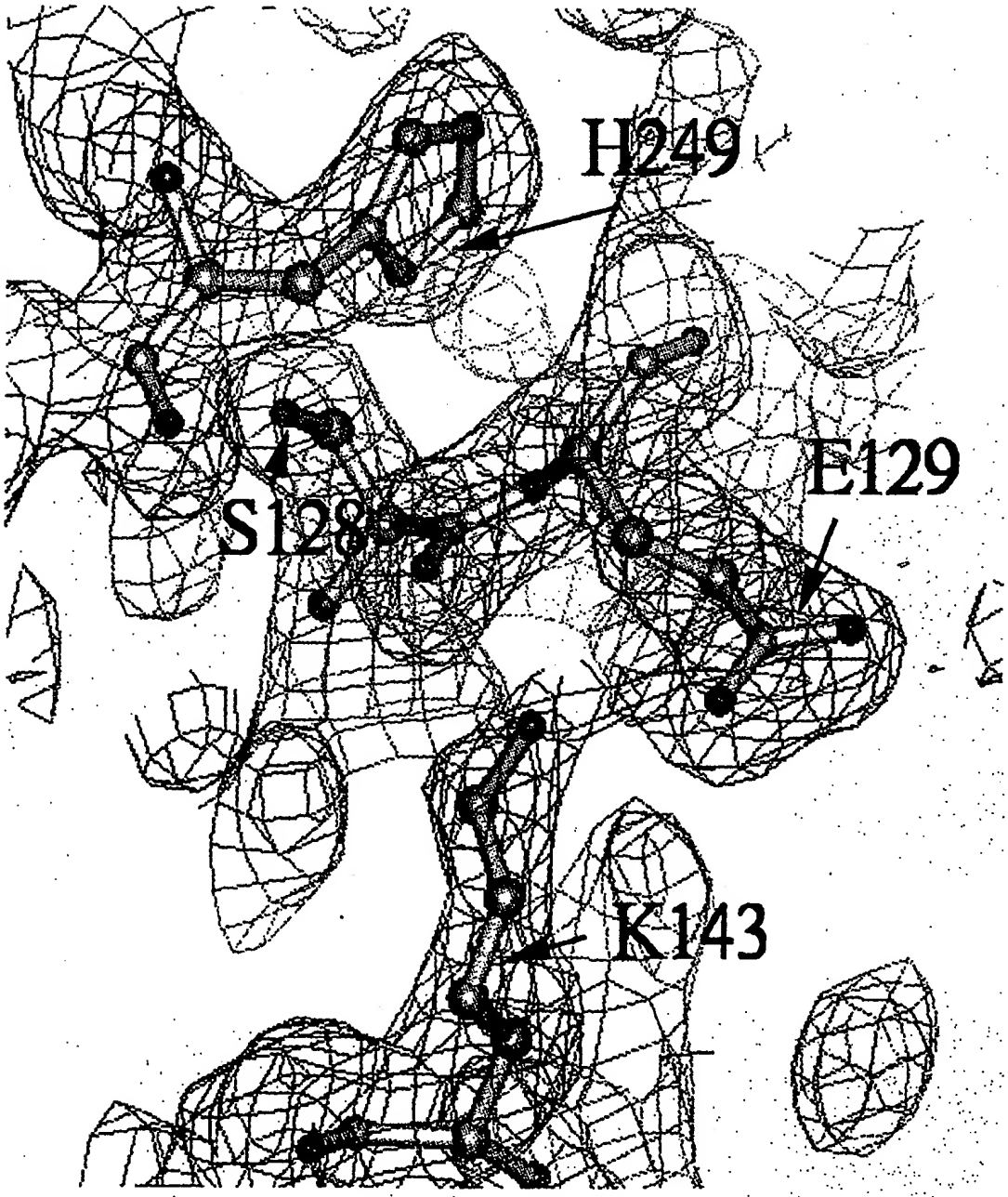

FIG. 1

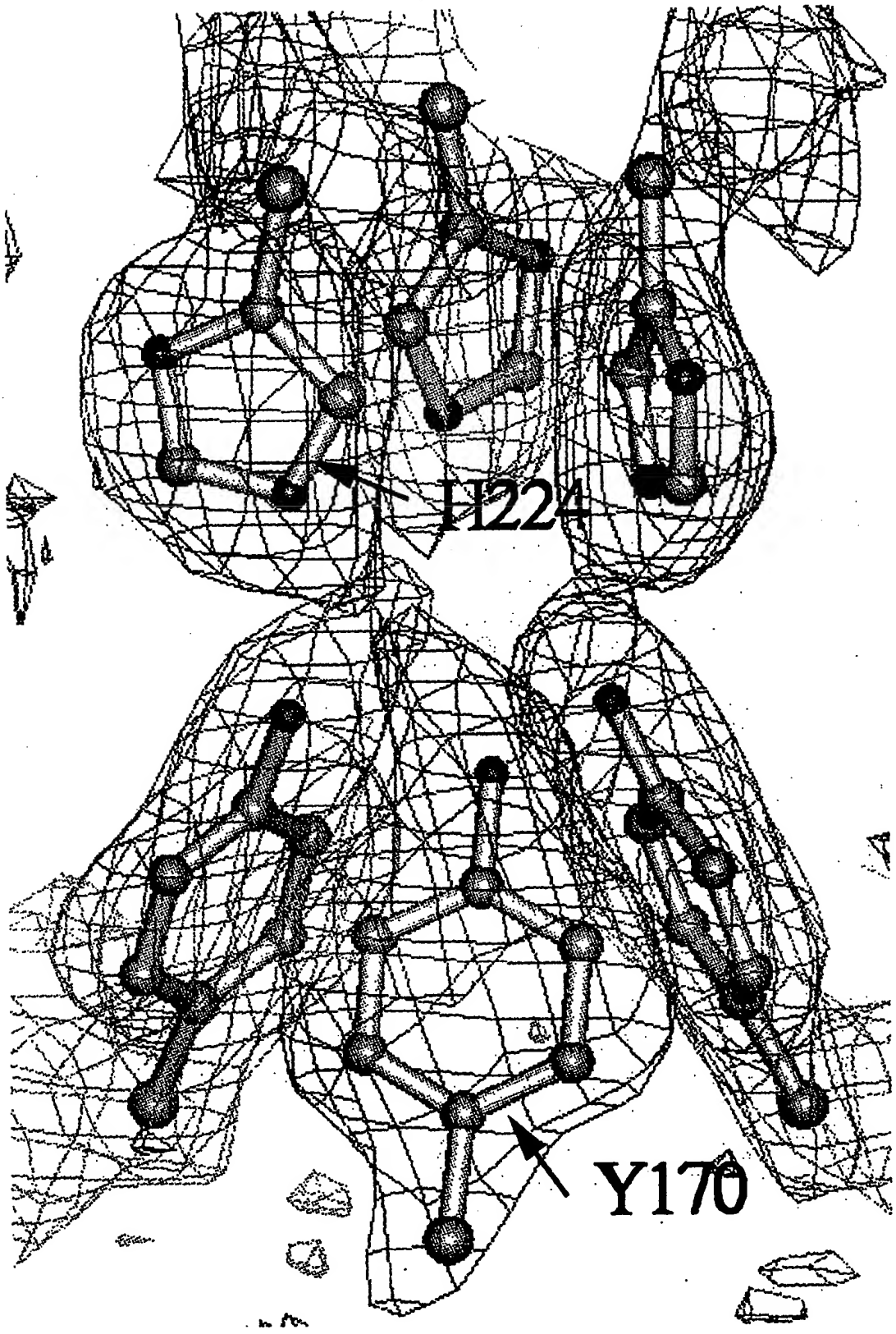

FIG. 2

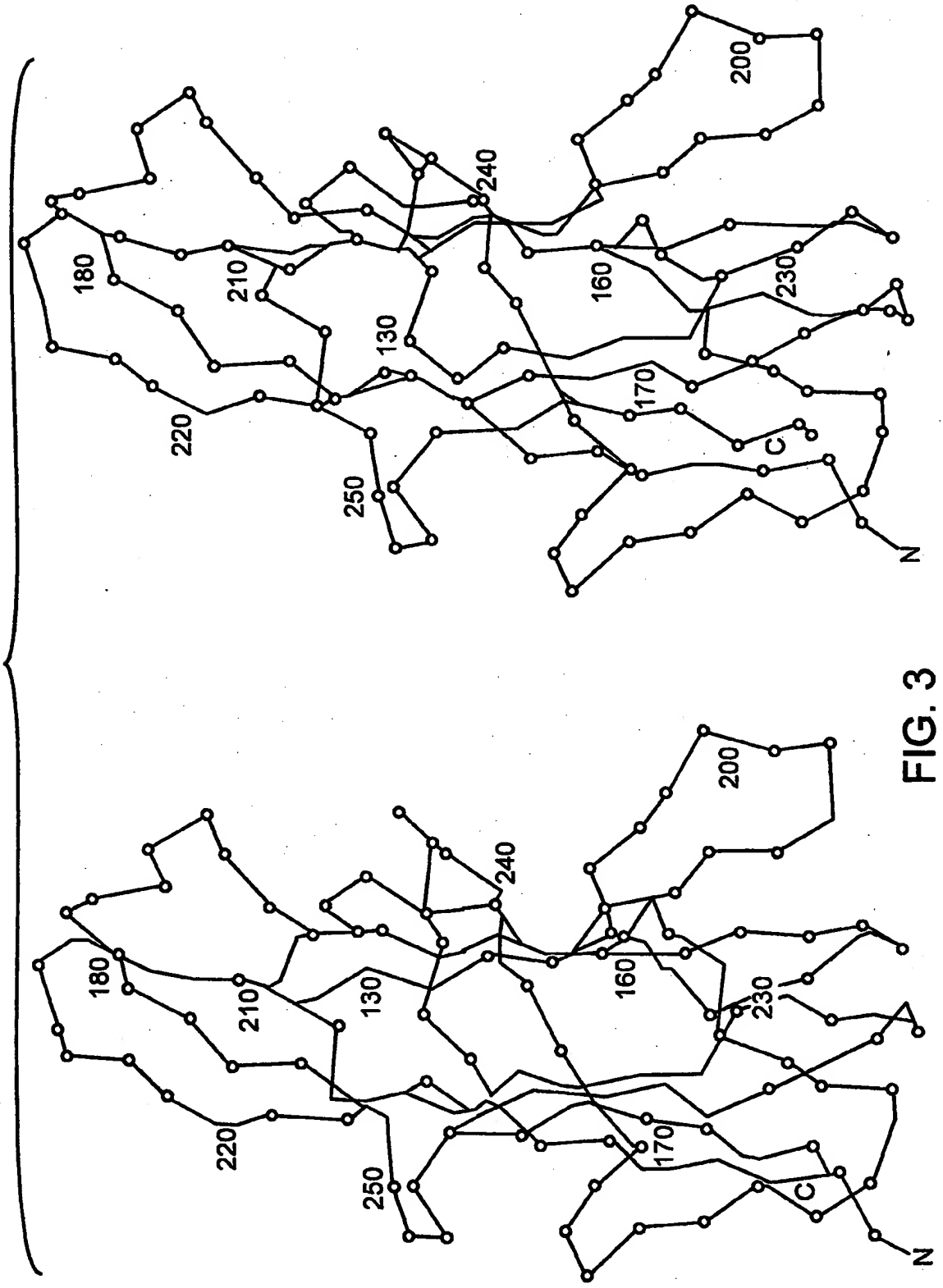
200
240
180
210
160
230
130
170
220
C
250
N
200
240
180
210
160
230
130
170
220
C
250
N

FIG. 3

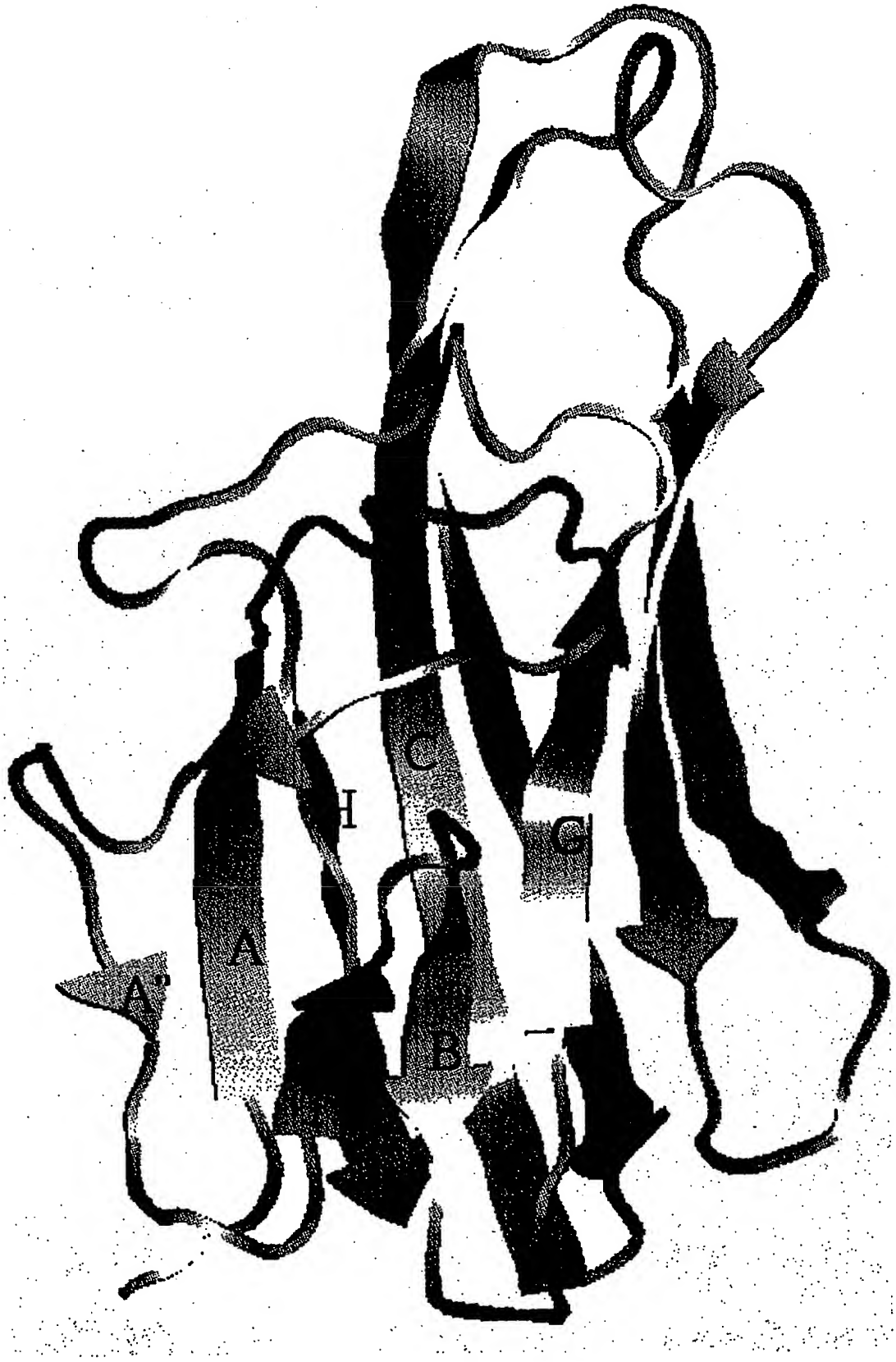

FIG. 4

```
              ---A--          --A"--      ----B'----    --B-
       115       125        135         145         155
TNFa:RTPSDKP   VAHVVANPQAEGQ   LQWLNRRANALLANGV ELRD NQLVVP
LTa:    TLKP   AAHLIGDPSKQNS   LLWRANTDRAFLQDGF SLSN NSLLVP
CD40L: GDQNPQIAAHVISEASSKTTSVLQW AEKGYYTMSNNLVTLENGKQLTVK
(1)                 *  ** *        ********
(2)     .......-..-.--.+...+....-...---......-.....

  -----C-----------          ----D----        -----E----
165       175             185        195          205
SEGLYLIYSQVLFKGQGCP     STHV LLTHTISRIAVSYQTKVNLLSAIKSPCQR
TSGIYFVYSQVVFSGKAYSPKATSSPL YLAHEVQLFSSQYPFHVPLLSSQKMVY
RQGLYYIYAQVTF     CSNREASSQAPFIASLCLKSPGRFE RI LLRAANTHS S
              ***          **  *          ***** *      * *
.....................+.....................++..........-....

    -----F-------------     --G---                    ---H---
215               225        235        245            255
ETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLLFAESGQVY  FGIIAL
  PGLQE   PWLHSMYHGAAFQLTQGDQLSTHTDGIPHLVLSPST VF  FGAFAL
AKPCGQ       QSIHLGGVFELQPGASVFVNVTDPSQV  SHGT GFTSFGLLKL
     *    * *                      * *  ***** *
.....................-.......-....+..+.....................
```

FIG. 5

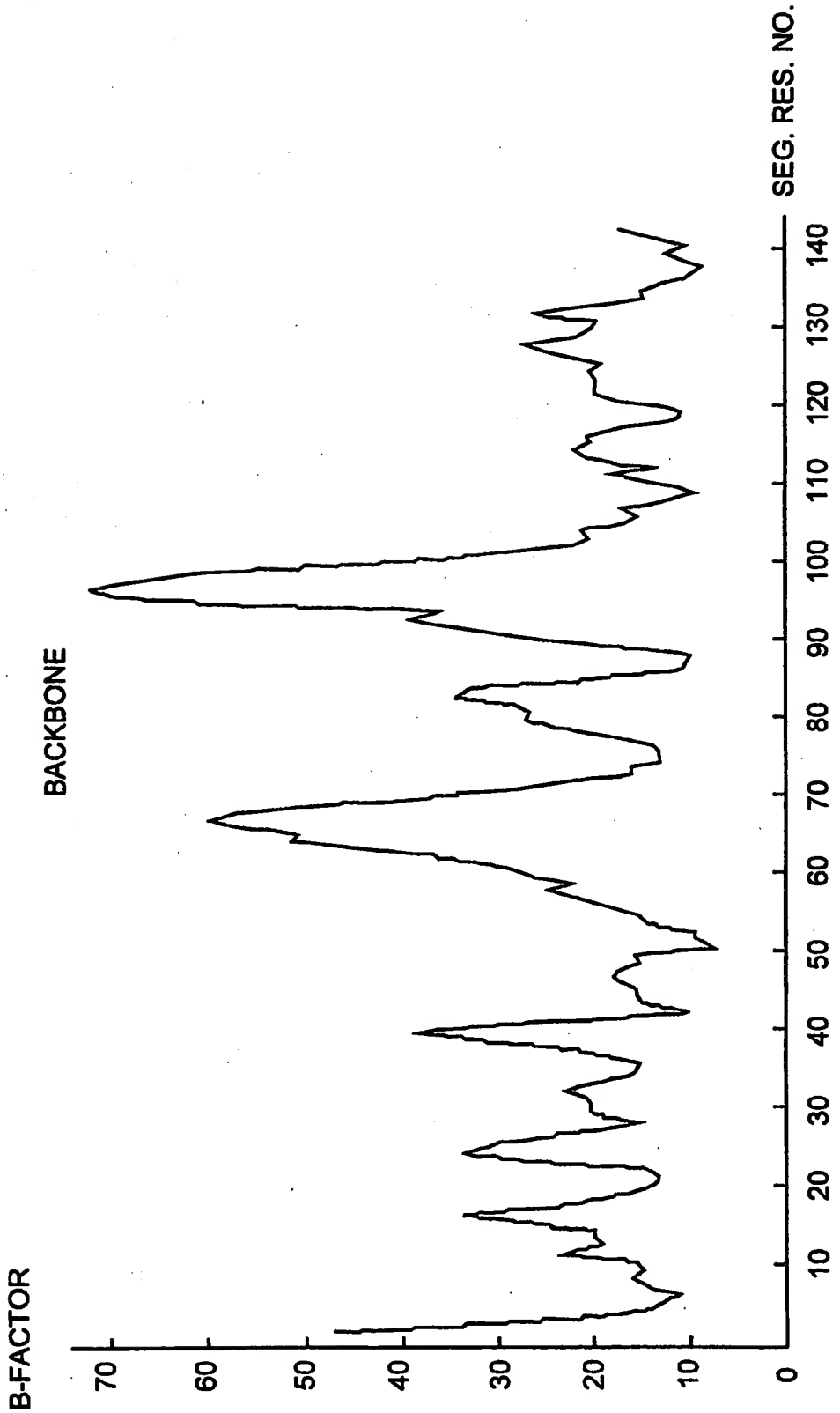
B-FACTOR
BACKBONE
SEG. RES. NO.
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140

FIG. 6

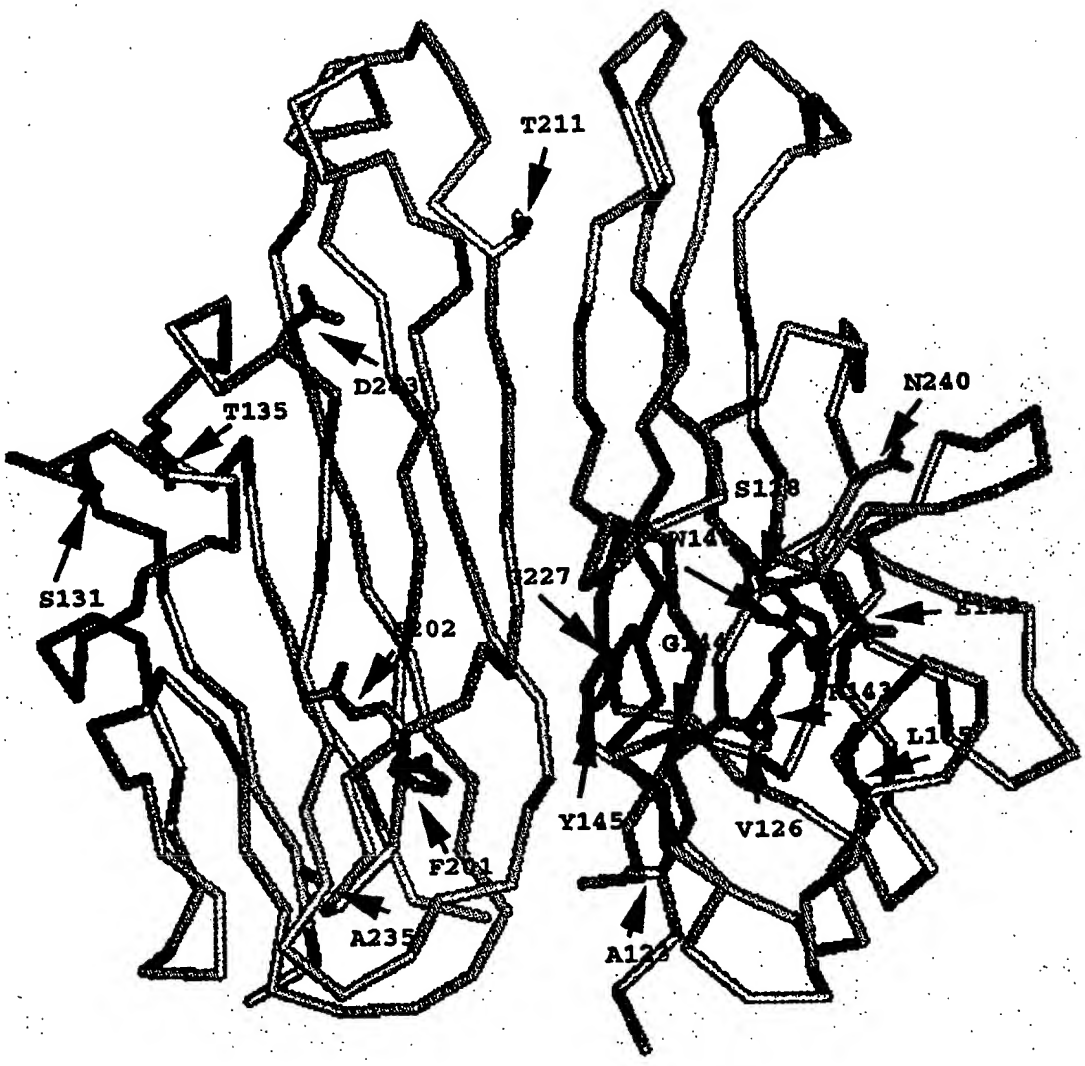

FIG. 7

CRYSTALLOGRAPHIC AND REFINEMENT DATA:

SYMMETRY:	R3
UNIT CELL:	a = b = 77.17 Å, c = 90.46 Å, $\alpha = \beta = 90°$, $\gamma = 120°$
NO. OF CRYSTALS:	1
RESOLUTION:	1.75 Å
REFLECTIONS (MEASURED):	42,587
REFLECTIONS (UNIQUE):	15,693
R_{MERGE}a:	6.7%
COMPLETENESS (OVERALL):	77.1%
COMPLETENESS (TO 2.1Å):	89.9%
R_{WORK}b:	21.8% FOR 7.5-2 Å DATA
R_{FREE}b:	29.1%
R.M.S DEVIATIONS	
BONDS:	0.021 Å
ANGLES:	3.9°

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a) $R_{sym} = \Sigma|I_{obs} - \langle I \rangle| / \Sigma \langle I \rangle$

b) $R_{free} = R_{work} = \Sigma|F_{obs} - F_{calc}| / \Sigma F_{obs}$

"FREE" AND "WORK" REFER TO THE FREE AND WORKING DATA SETS, RESPECTIVELY.

FIG. 8

INTERNATIONAL SEARCH REPORT

Inter ''onal Application No
PCT/US 96/10664

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/725 C07K14/525 C07K1/00 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 585 943 (BRISTOL-MYERS SQUIBB COMPANY) 9 March 1994 see the whole document ---	21-26,36
X	EP,A,0 555 880 (BRISTOL-MYERS SQUIBB COMAPNY) 18 August 1993 see the whole document ---	21-26,36
X	WO,A,94 17196 (IMMUNEX COPRPORATION) 4 August 1994 see the whole document ---	21-26,36
X	WO,A,93 08207 (IMMUNEX CORPORATION) 29 April 1993 see the whole document --- -/--	21-26,36

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 1996	15. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Masturzo, P

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INTERNATIONAL SEARCH REPORT

Inter ··onal Application No
PCT/US 96/10664

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGICAL METHODS, vol. 149, no. 2, 15 June 1992, NEW YORK US, pages 655-660, XP002017167 W C FANSLOW ET AL.: "Soluble forms of CD40 inhibit biologic responses of human B cells" see the whole document	21-26,36
X	CHEMICAL ABSTRACTS, vol. 120, no. 3, 17 January 1994 Columbus, Ohio, US; abstract no. 28892n, M C PEITSCH & V C JONGENEEL: "A 3-D model for the CD40 ligand predicts that it is a compact trimer similar to the tumor necrosis factor" page 659; XP002017169 see abstract & INT. IMMUNOL. , vol. 5, no. 3, 1993, pages 233-238,	13, 21-26, 28-38
P,X	BIOCHEMISTRY, vol. 34, no. 31, 8 August 1995, EASTON, PA US, pages 9884-9892, XP002017168 J BAJORATH ET AL.: "Analysis of gp39/CD40 interactions using molecular models and site-directed mutagenesis" see the whole document	13, 21-26, 28-38
P,X	CHEMICAL ABSTRACTS, vol. 123, no. 23, 4 December 1995 Columbus, Ohio, US; abstract no. 311922s, M KARPUSAS ET AL.: "2A crystal structure of an extracellular fragment of human CD40 ligand" page 700; XP002017170 see abstract & STRUCTURE, vol. 3, no. 10, 1995, LONDON, pages 1031-1039,	1-38

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INTERNATIONAL SEARCH REPORT

nformation on patent family members

Inter "onal Application No
PCT/US 96/10664

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-585943	09-03-94	US-A-	5540926	30-07-96
		AU-A-	4612093	10-03-94
		CA-A-	2105552	05-03-94
		FI-A-	933862	05-03-94
		HU-A-	69977	28-09-95
		JP-A-	6315383	15-11-94
		NO-A-	933126	07-03-94
		NZ-A-	248569	26-10-95
		ZA-A-	9306491	25-03-94
EP-A-555880	18-08-93	AU-A-	3298893	19-08-93
		CA-A-	2089229	15-08-93
		JP-A-	6220096	09-08-94
		NZ-A-	245898	27-04-95
		ZA-A-	9301013	20-09-93
WO-A-9417196	04-08-94	US-A-	5565321	15-10-96
		AU-A-	6231394	15-08-94
		CA-A-	2153806	04-08-94
		EP-A-	0679191	02-11-95
WO-A-9308207	29-04-93	AU-B-	661360	20-07-95
		AU-A-	3122693	21-05-93
		CA-A-	2121798	29-04-93
		EP-A-	0667901	23-08-95
		FI-A-	941837	30-05-94
		JP-T-	7504083	11-05-95
		NO-A-	941422	27-06-94